

# PRODUCT CATALOG 2012



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## Table of Contents

<b>Section 1: Section 1: Ultra Sensitive Luminescence Kits</b> .....	<b>1</b>
Non-Radioactive Cytotoxicity Assay – aCella™ - TOX.....	2
Non-Radioactive Assay for Acetylcholinesterase Activity – aCella™ - AChE.....	4
Cyclic AMP Chemiluminescent Immunoassay Kit – aCella™ - cAMP .....	6
Cyclic GMP Chemiluminescent Immunoassay Kit – aCella™ – cGMP.....	8
<b>Section 2: Apoptosis / Caspase Detection Kits</b> .....	<b>10</b>
High Throughput Screen for Caspase 3/7 Detection – APO HTSTM .....	11
Active Caspase Detection (1 – 10) in Live Cells – APO LOGIX™ .....	13
Sulforhodamine-Based Caspase Detection Kits .....	15
Dual Sensor Caspase and Mitochondria Membrane Potential Detection Kits – MitoCasp™ .....	17
Antibody-Specific Caspase 3 Detection Kits – Apo Active 3™ FITC.....	20
Antibody-Specific Caspase 3 Detection Kits – Apo Active 3™ PE.....	22
Apoptosis Induced DNA Damage Assay - ApossDNATM .....	24
Apoptosis Induced DNA Damage & Caspase 3 Assay - DNACASP3™ .....	26
<b>Section 3: Apoptosis / Mitochondria Membrane Potential Detection</b> .....	<b>27</b>
Mitochondrial Membrane Potential Kit – JC 1.....	28
Mitochondrial Membrane Potential Detection – Mito Flo .....	31
<b>Section 4: Enzymatic Assays</b> .....	<b>33</b>
Fluorescent Monoamine Oxidase Detection Assay Kit – Fluoro MAOTM .....	34
Fluorescent Semicarbazide-Sensitive Amine Oxidase Detection Assay Kit – Fluoro SSAOTM .....	36
Fluorescent Catalase Detection Assay Kit – Fluoro Catalase™ .....	38
Myeloperoxidase Detection Kit – Fluoro MPOTM .....	40
AChE Detection in RBC, Saliva, lysates – Fluoro AChETM .....	42
Chlorination and Peroxidation Detection – Fluoro MPOHOCL™ .....	44
Eosinophil peroxidase Detection Kit - Fluoro EPOTM .....	46
<b>Section 5: Metabolic Assays</b> .....	<b>48</b>
Fluorescent Thiol Detection Kit - Fluoro Thiol™ .....	49
Fluorescent NAD/NADH Detection Kit - Fluoro NAD/NADHTM .....	51
Fluorescent NADP/NADPH Detection Kit - Fluoro NADPHTM.....	53
Total Cholesterol Detection Kit - Fluoro Cholesterol™.....	55
Fluorescent Sarcosine Detection Kit - Fluoro Sarcosine™ .....	57
<b>Section 6: Cytotoxicity Using Flow Cytometry</b> .....	<b>58</b>
Non Radioactive Assay for Cell / Antibody Mediated Cytotoxicity – ACT 1.....	59
<b>Section 7: Oxidative Stress Detection</b> .....	<b>61</b>
Hydrogen Peroxide Detection Kit – Fluoro H <sub>2</sub> O <sub>2</sub> ™.....	63
Nitric Oxide Synthase Detection Kit – NOS Detection.....	65
Fluorescent Hypochlorite (OCI-) Detection Kit.....	67
Fluorescent Hydroxyl (OH) / Peroxynitrite (ONOO-) Detection Kit .....	69
Fluorescent hROS Detection Kit – Fluoro hROSTM.....	71
Colorimetric Super Oxide Dismutase Detection Kit - SOD.....	73
Glutathione S-Transferase Fluorescence Activity Kit - Fluoro GST™ .....	75
<b>Section 8: Ultra pure chemicals</b> .....	<b>77</b>
Ultra Pure grade Adenosine 5'-diphosphate – Ultra Pure ADP .....	78

## Table of Contents (Cont'd)

<b>Section 9: Coating Buffers and ELISA Diluents .....</b>	<b>79</b>
Block Buffers.....	80
Assay Diluents .....	81
Universal Plate Coating Buffers.....	82
Conjugate Diluents.....	83
Sample Diluents.....	85
Wash Buffers.....	86
<b>Section 10: Contract Research Services.....</b>	<b>87</b>
ADCC / CDC Assay Services .....	88

# INTRODUCTION

Cell Technology, Inc. - Product Catalog 2012

## Introduction

In this catalog you will find a complete listing of all cellular study products currently offered by Cell Technology, Inc. Included in the listings are key benefits, technological information, and prices for each of the products. In some cases, figures are provided to show the product's action, expected results, and other information, depending on the complexity of the product.

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# Section 1

## **Section 1: Ultra Sensitive Luminescence Kits**

# Non-Radioactive Cytotoxicity Assay – aCella™ - TOX

## Key Benefits

The Key Benefits of our aCella™ - TOX are as follows:

- Safe - Non Radioactive Enzyme release assay
- Versatile - Assay can be run in serum supplemented media
- Homogenous - One-step, no wash assay. Assay can be run in same plate as samples
- Fast - Results in 3-5 minutes
- Highly Sensitive - Can detect less than 500 cells/well
- HTS - Adaptable for High Throughput format
- Non-destructive - Assay allows monitoring of additional parameters

## Introduction to aCella™ - TOX

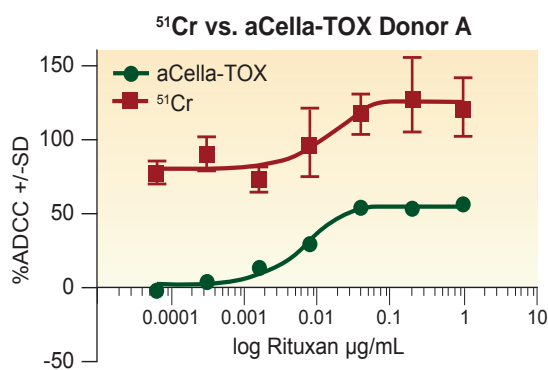
Cell Technology introduces aCella™ - TOX, a new and highly sensitive assay using its recently patented Coupled Luminescent technology for the detection of cytotoxicity. This assay is designed to quantitatively measure the release of Glyceraldehyde-3-Phosphate Dehydrogenase (G3PDH) from mammalian cell lines or bacterial cells (1, 2, 3, 4).

Other enzyme release assays, for example the Lactate Dehydrogenase (LDH) release assay (5, 6, 7, 8), suffer from low sensitivity as a result of interference by serum or phenol red present in the media. aCella™ - TOX can work in both these media formulations and allowing overnight assays while retaining sensitivity.

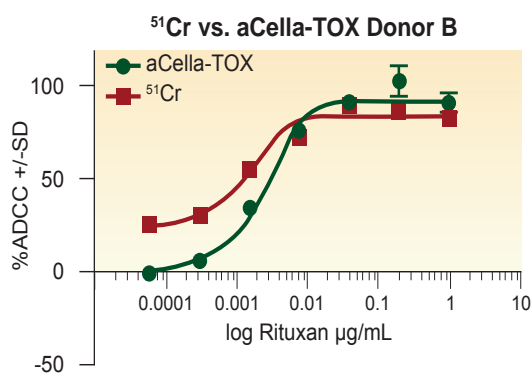
## Assay Principle

G3PDH is an important enzyme in the glycolysis and gluconeogenesis pathways. This homotetrameric enzyme catalyzes the oxidative phosphorylation of D-glyceraldehyde-3-phosphate to 1, 3-diphosphoglycerate in the presence of cofactor and inorganic phosphate.

The release of G3PDH is coupled with the enzyme 3-Phosphoglyceric Phosphokinase (PGK) to produce ATP. ATP is detected via the luciferase, luciferin Bioluminescence methodology. Further, ATP is detected with aCella™ - TOX as a homogenous assay that can measure cytotoxicity or cell viability in the same plate. Additionally, supernatants can be removed from the original assay plate and assayed in a different plate, allowing kinetic assays to be set up. A further feature is that the assay is non-destructive which allows monitoring of additional parameters such as gene expression.



*Donor A: A Direct comparison of <sup>51</sup>Cr and aCella-TOX was carried out with the same donors with Daudi Cells. Log (EC50) value for aCella-Tox was -2.23, and that for <sup>51</sup>Cr was -2.087 for Donor A.*



*Donor B: A Direct comparison of <sup>51</sup>Cr and aCella-TOX was carried out with the same donors with Daudi Cells. Log (EC50) value for aCella-Tox was -2.612, and that for <sup>51</sup>Cr was -2.77 for Donor B.*

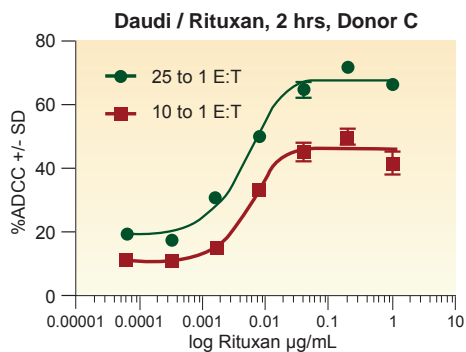


Figure 3: 5000 Daudi cells/well were incubated with serially diluted Rituxan antibody for 15 minutes prior to the addition of purified NK cells stimulated overnight with IL-2. The ADCC reaction was further incubated for 2 hours at the specified E:T ratios. % ADCC was measured using the aCella™ - TOX assay.

## Kit Contents

The aCella™ - TOX Kit includes the following:

- Part #3008: Enzyme Assay Diluent
- Part #3009: 5.5x Detection Assay Diluent
- Part #6001: Lyophilized Enzyme Assay Reagent
- Part #6002: 50x Detection Reagent
- Part #6003: Glyceraldehyde 3-Phosphate (G3P)
- Part #3035: Lysis Buffer

## Ordering Information

The following aCella™ - TOX Kits are available:

Catalog No	Quantity	Price (in US dollars)
CLATOX 100-3	500 Tests	\$695
CLATOX 100-4	1,000 Tests	\$1345

**NOTE:** Volume discounts are available. Please call for pricing.

## References

Methods and compositions for coupled luminescent assays. United States Patent 6,811,990 Corey, et al. November 2, 2004

Corey et al, *J. Immunol. Methods*, vol. 207(1), Aug. 22, 1997, pp. 43-51.

Corey, M.J., et al., "A Very Sensitive Coupled Luminescent Assay for Cytotoxicity and Complement-Mediated Lysis," *Journal of Immunological Methods* 207:43-51, 1997.

Crouch, S.P.M., et al., "The Use of ATP Bioluminescence as a Measure of Cell Proliferation and Cytotoxicity," *Journal of Immunological Methods* 160:81-88, 1993.

Schafer, H., et al., "A Highly Sensitive Cytotoxicity Assay Based on the Release of Reporter Enzymes, From Stably Transfected Cell Lines," *Journal of Immunological Methods* 204:89-98, 1997.

Racher, LDH Assay, in *Cell and tissue culture: Laboratory procedures in biotechnology*, A. Doyle and J.B. Griffiths, Eds. 1998, *John Wiley & Sons: Chichester, New York, Weinheim*. p. 71-5.

Decker, T. and Lohmann-Matthes, M.L. (1988) A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity. *J. Immunol. Meth.* 115, 61-9.

Korzeniewski, C. and Callewaert, D.M. (1983) An enzyme-release assay for natural cytotoxicity. *J. Immunol. Meth.* 64, 313-20.

## Non-Radioactive Assay for Acetylcholinesterase Activity – aCella™ - AChE

### Key Benefits

The Key Benefits of our the Non-Radioactive Assay for Acetylcholinesterase Activity – aCella™ - AChE product are as follows:

- Safe – Non-radioactive enzyme release assay
- Versatile - Nerve gas, pesticide monitoring, drug screening Aplns
- Homogenous - One-step, no wash assay
- Fast - Results in 30 seconds -5 minutes
- Highly Sensitive
- HTS - Adaptable for High Throughput format
- Standard luminometer readout

### Introduction to aCella™ – AChE

Acetylcholinesterase (AChE) is one of the most important enzymes involved in nerve transmission. The enzyme is bound to cellular membranes of excitable tissue (synaptic junction, endoplasmic reticulum, etc) (1-3). Acute toxicity to humans and animals through inhibition of AChE by both nerve gases and an important class of pesticides has long been a field of intensive scientific investigation (4, 5). AChE inhibitors have also been used clinically as Alzheimer's treatments (e.g., tacrine (tetrahydroaminoacridine)) (6) and are the subject of increasing interest in various disease processes and treatment strategies (7, 8). However, both environmental detection of AChE inhibitors and development of modulators of AChE enzymatic activity as drugs have been hampered by the difficulty and complexity of the current assay methods.

### Assay Principle

We have developed a highly sensitive, very rapid, extremely simple assay for monitoring AChE activity, using the natural substrate, acetylcholine. A series of coupled enzyme reactions quickly translates the presence of active AChE into a change in the luminance of the reaction. First, acetylcholine is hydrolyzed by the AChE to yield acetate and choline. The acetate is then phosphorylated via acetate kinase, resulting in consumption of ATP, and finally the ATP concentration is measured by the well established luciferase method. These reactions can occur simultaneously, and the result is generally obtained in five minutes or less. Inhibitors of AChE are readily detected by an increase in luminance due to reduced consumption of ATP.

The following reaction illustrates the sequence of events if AChE inhibitors are present:

Reaction I: AChE + Inhibitor  $\xrightarrow{\text{X}}$  No Acetate and Choline.

Reaction II: Coupled Enzyme Reaction + ATP  $\xrightarrow{\text{X}}$  Reaction does not proceed.

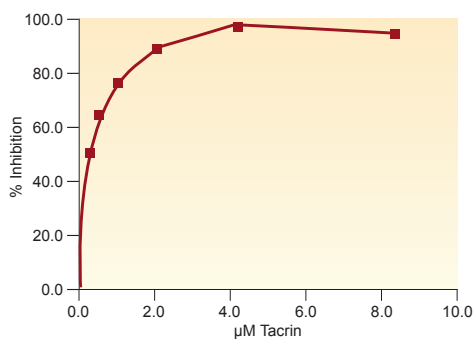


Figure A. Tacrine (a mixed-mode inhibitor of AChE) was serially diluted in DI water. Next 10mL of the diluted Tacrine (x axis labeling represents mM final concentration of Tacrine) was added to a white opaque 96-well microplate along with 50 mL of component A (AChE enzyme). The samples were incubated for 5 minutes after which 50mL of component B was added to all the wells. Data were collected using a luminometer. Data shown represents T=2 minutes after the addition of component B.

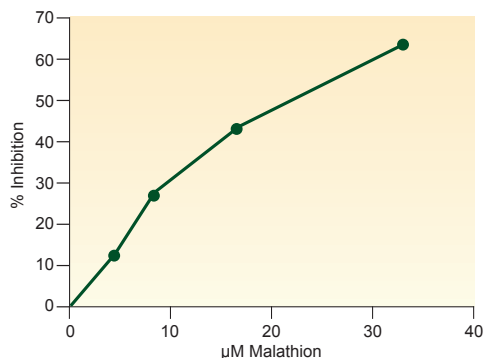


Figure B. Malathion, a common pesticide, was first diluted in DMSO and subsequently serially diluted in Di water. 10mL of the diluted Malathion (x axis represents mM final concentration of Malathion) was added to a white opaque 96-well microplate followed by 50 mL of component A (AChE enzyme). The mixture was incubated for 15 minutes, after which 50 mL of component B was added to all the wells. Data were collected using a luminometer. Data shown is at T= 5 minutes. Data shown represents T=2.5 minutes after the addition of component B.

Reaction III: ATP (remaining) + Luciferase/Luciferin  $\longrightarrow$  LIGHT

### Kit Contents

The aCella™ - AChE Kit includes the following:

- Part #3023: Component A: Contains acetylcholinesterase
- Part #3024: Component B: Contains detection reagent, acetylcholine and kinase enzymes
- Part # 3025: Control to measure maximum luminescence

### Ordering Information

The following acella™ - AChE Kit is available

Catalog No	Quantity	Price (in US dollars)
CLACHE 100-2	100 Tests	\$395
CLACHE 100-3	500 Tests	\$1295
CLACHE 100-4	1000 Tests	\$2195

**NOTE:** Volume discounts are available. Please call for pricing.

### References

Polittoff, A., Blitz, A., and Rose, S.: Incorporation of Acetylcholinesterase Into Synaptic Vesicles is Associated with Blockade of Synaptic Transmission, *Nature* 256, 324, 1975

Friedenberg, R., and Seligman, A.: Acetylcholinesterase at the Myoneural Junction: Cytochemical Ultrastructure and Some Biochemical Considerations, *J Histochem Cytochem* 20, 771, 1972

Nachmansohn, D.: Proteins in Excitable Membranes, *Science* 168, 1059, 1970.

HA Berman and MM Decker. Kinetic, equilibrium, and spectroscopic studies on dealkylation ("aging") of alkyl organophosphonyl acetylcholinesterase. Electrostatic control of enzyme topography. *J. Biol. Chem.*, Aug 1986; 261: 10646-10652.

Arie Ordentlich et al. The Architecture of Human Acetylcholinesterase Active Center Probed by Interactions with Selected Organophosphate Inhibitors. *J. Biol. Chem.*, May 1996; 271: 11953-11962.

Levy R. Tetrahydroaminoacridine and Alzheimer's disease. *Lancet*, 1987 Feb 7; 1(8528):322.

Bolognesi ML et al. Propidium-based polyamine ligands as potent inhibitors of acetylcholinesterase and acetylcholinesterase-induced amyloid-beta aggregation. *J Med Chem*. 2005 Jan 13; 48(1):24-7.

(8) Schallreuter KU et al. Activation/deactivation of acetylcholinesterase by H2O2: more evidence for oxidative stress in vitiligo. *Biochem Biophys Res Commun*. 2004 Mar 5; 315(2):502-8.

## Cyclic AMP Chemiluminescent Immunoassay Kit - aCella™ - cAMP

### Key Benefits:

The Key Benefits of our aCella™ - cAMP Detection Kits are as follows:

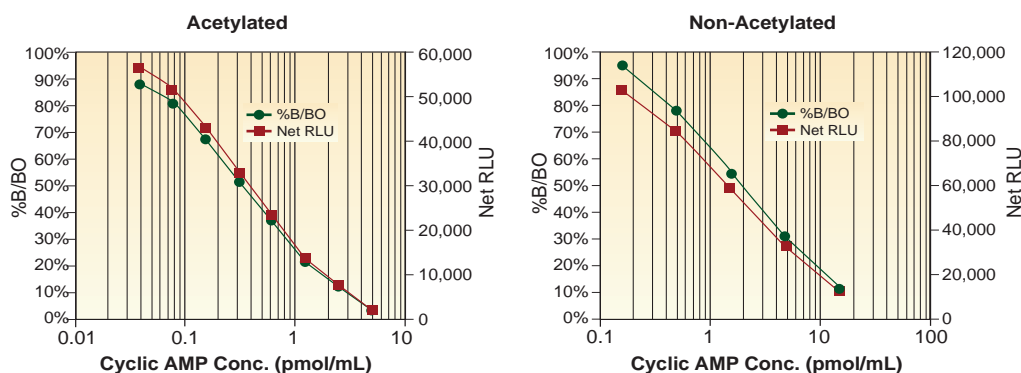
- Ultra-sensitive – Measures as little as < 1 fmol cAMP per sample
- Fast – Rapid assay, results in 2 hours
- Adaptable – Assay can be used with 96 or 384 –well plates
- Convenient – Suitable for use in multiple sample matrices

### Description:

Cyclic AMP (cAMP) is one of the most important secondary messengers and a key intracellular regulator. Discovered by Sutherland and Rall in 1957, it functions as a mediator of activity for a number of hormones, including epinephrine, glucagon, and ACTH. Cyclic AMP is produced by the enzyme adenylate cyclase, and the enzyme is activated by the hormones glucagon and adrenaline and by G protein. cAMP decomposition into AMP is catalyzed by the enzyme phosphodiesterase. Other biological actions of cAMP include regulation of innate immune functioning, axon regeneration, cancer and inflammation

The aCella™ – cAMP Chemiluminescent Immunoassay kit is designed to quantitatively measure cAMP present in cell lysates, plasma, urine, saliva, tissue and culture media samples. The supplied Sample Diluent will lyse cells, stabilize cAMP and stop phosphodiesterase activity. A cAMP standard is provided to generate a standard curve for the assay. The supplied Plate Primer solution is added to the wells of a coated white microtiter plate, followed by standards or diluted samples. A cAMP-peroxidase conjugate is then added to the wells.

The binding reaction is initiated by the addition of a polyclonal antibody to cAMP. After a 2-hour incubation, the plate is washed and chemiluminescent substrate is added. The substrate immediately reacts with the bound cAMP-peroxidase conjugate. The generated chemiluminescent glow signal is measured. The concentration of the cAMP in the sample is calculated, after making correction for the dilution.



### Kit Contents

The aCella™ - cAMP Kit includes the following:

- |                                           |                                  |                                        |
|-------------------------------------------|----------------------------------|----------------------------------------|
| • Part #90013: Plate Sealer               | Concentrate                      | • Part #90022: Triethylamine           |
| • Part #90014: Coated White 96 Well Plate | • Part #90018: Conjugate Diluent | • Part #90023: Wash Buffer Concentrate |
| • Part #90015: Cyclic AMP Standard        | • Part #90019: Sample Diluent    | • Part #90024: Substrate Solution A    |
| • Part #90016: Cyclic AMP Antibody        | • Part #90020: Plate Primer      | • Part #90015: Substrate Solution B    |
| • Part #90017: Cyclic AMP Conjugate       | • Part #90021: Acetic Anhydride  |                                        |

**Ordering Information:**

Catalog No	Size	Price (in US dollars)
CLACAMP 100-2	100 Tests	\$375

**References:**

Sutherland, E. W. and Rall, T. W. Fractionation and Characterization of a Cyclic Adenine Ribonucleotide Formed by Tissue Particles. *J. Biol. Chem.*, 232:1077, 1958.

Marsh, J.M., The Role of Cyclic AMP in Gonadal Steroidogenesis. *Biol. Reprod.*, 14:30-53,1976.

Korenman, S.G. and Krall, J.F., The Role of Cyclic AMP in the Regulation of Smooth Muscle Cell Contraction in the Uterus. *Biol. Reprod.*, 16:1-17, 1977.

Kelley, D.J., Bhattacharyya, A., Lahvis, G.P., Yin, J.C.P., Malter, J., and Davidson, R.J., The Cyclic AMP Phenotype of Fragile X and Autism. *Neurosci. Biobehav. Rev.*, 32(8): 1533-1543, 2008.

<http://www.hmdb.ca/metabolites/HMDB00058>

Serezani, C.H., Ballinger, M.N., Aronoff, D.M., and Peters-Golden, M., Cyclic AMP. Master Regulator of Innate Immune Cell Function. *Am. J. Resp. Cell and Mol. Biol.*, 39 (2): 127, 2008.

Hannila, S.S., and Filbin, M.T., The role of cyclic AMP signaling in promoting axonal regeneration after spinal cord injury. *Exp. Neurol.*, 209(2): 321–332, 2008.

Shankar, D.B, Cheng, J.C., and Sakamoto, K.M., Role of cyclic AMP response element binding protein in human leukemias. *Cancer*, 104(9): 1819–24, 2005.

Galea E. and Feinstein, D.L., Regulation of the expression of the inflammatory nitric oxide synthase (NOS2) by cyclic AMP. *FASEB J.*, 13:2125- 2137, 1999.

NIH Clinical Center, <http://cclnprod.cc.nih.gov/dlm/testguide.nsf/Index/EB6E90F8D951346F85256B A4004C96E4?OpenDocument>

NIH Clinical Center, <http://cclnprod.cc.nih.gov/dlm/testguide.nsf/Index/24B381AEE513EB8785256B A40052ADAD?OpenDocument>

Sproles, A.C., Cyclic AMP Concentration in Saliva of Normal Children and Children with Down's Syndrome, *J. Dent. Res.*, 1976, 52, 915-917.

## Cyclic GMP Chemiluminescent Immunoassay Kit - aCella™ - cGMP

### Key Benefits:

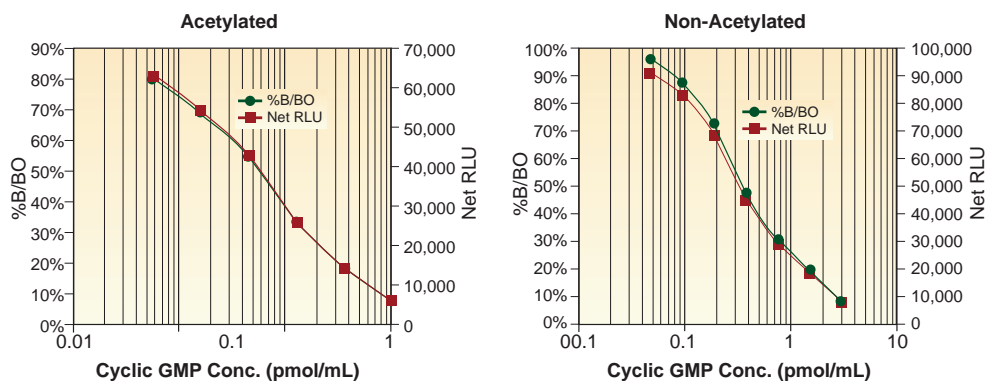
The Key Benefits of our aCella™ - cAMP Detection Kits are as follows:

- Ultra-sensitive – Measures as little as < 1 fmol cGMP per sample
- Flexible – One kit for Extra- and Intra-Cellular cGMP quantification
- Convenient – Stable, 4°C liquid reagents
- Ready-to-use – Complete kit with validated Cell Lysis Reagent Included

### Description:

Cyclic GMP is a critical and multifunctional secondary messenger present at levels typically 10-100 fold lower than cAMP in most tissues. Intracellular cGMP is formed by the action of the enzyme guanylate cyclase on GTP and degraded through phosphodiesterase hydrolysis. Guanylate cyclases (GC) are either soluble or membrane bound. Soluble GCs are nitric oxide responsive, whereas membrane bound GCs respond to hormones such as acetylcholine, insulin and oxytocin. Other chemicals like serotonin and histamine also cause an increase in cGMP levels. Cyclic GMP regulates cellular composition through cGMP-dependent kinase, cGMP-dependent ion channels or transporters and through its hydrolytic degradation by phosphodiesterase.

The aCella™ – cGMP Chemiluminescent Immunoassay kit measures cGMP present in lysed cells, EDTA, plasma, urine, saliva and culture media samples. The kit is unique in that all samples and standards are diluted into an acidic Sample Diluent, which contains special additives and stabilizers, for cGMP measurement. Acidified samples of cGMP are stable and endogenous phosphodiesterases are inactivated in the Sample Diluent. After an overnight incubation at 4°C, the plate is washed and the chemiluminescent substrate is added. The substrate generates light that is detected in a multilabel microtiter plate reader capable of reading luminescence.



### Kit Contents

The aCella™ - cGMP Kit includes the following:

- Part #90001: Coated White 96 Well Plate
- Part #90002: Cyclic GMP Standard
- Part #90003: Cyclic GMP Antibody
- Part #90004: Cyclic GMP Conjugate Concentrate
- Part #90005: Conjugate Diluent
- Part #90006: Sample Diluent
- Part #90007: Plate Primer
- Part #90008: Acetic Anhydride
- Part #90009: Triethylamine
- Part #90010: Wash Buffer Concentrate
- Part #90011: Substrate Solution A
- Part #90012: Substrate Solution B
- Part #90013: Plate Sealer

**Ordering Information:**

Catalog No	Size	Price (in US dollars)
CLACGMP 100-2	100 Tests	\$395

**References:**

Domek-Lopacinska, K. and Strosznajder, JB. "Cyclic GMP metabolism and its role in brain physiology " (2005) *J Physiol Pharmacol* 56 Suppl 2, 15-34.

Lucas, K.A. et al. "Guanylyl cyclases and signaling by cyclic GMP " (2000) *Pharmacol Rev* 52: 375-414.

Ashman, DF, et al., "Isolation of adenosine 3', 5'-monophosphate and guanosine 3', 5'-monophosphate from rat urine." (1963), *Biochem Biophys Res Comm*, 11: 330-4.

Potter LR, Abbey-Hosch S, and Dickey DM. "Natriuretic peptides, their receptors, and cyclic guanosine monophosphate-dependent signaling functions". (2006) *Endocr Rev* 27: 47-72.

Waldman, SA and Murad, F., "Cyclic GMP synthesis and function " (1987) *Pharmacol Revs*, 39: 163-197.

Tremblay J, Gerzer R, and Hamet P., "Cyclic GMP in cell function". (1988) ,*Adv. 2nd Messenger & Phosphoprotein Res.*, 22: 319-383.

Matsumoto T, Kobayashi T, and Kamata K "Phosphodiesterases in the vascular system." (2003) *J Smooth Muscle Res* 39: 67-86.



# SECTION 2

## Section 2: Apoptosis / Caspase Detection Kits

## High Throughput Screen for Caspase 3/7 Detection – APO HTS

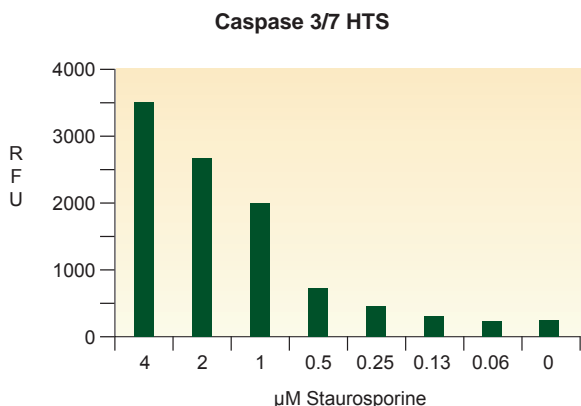
### Key Benefits

The Key Benefits of our the APO HTS™ Kit are as follows:

- Homogenous assay for active caspase 3/7.
- Breakthrough in cell lysis buffer and preservation of caspase activity
- Results in a no-wash, one-step assay
- No need to wash out media from cell samples, just add the reagent directly to your experimental samples.
- Easy to Use: No need to make cell lysates or run Western blots
- Works with suspension and adherent cells.

### Assay Principle

Cell Technology's APO 3/7 HTS™ Assay utilizes the quenched (z-DEVD)2-R110 peptide substrate for caspase 3/7 detection. The absorption and emission properties of the R110 dye are suppressed when attached to the z-DEVD peptide sequence. When R110 is cleaved away, by active caspase 3/7, from the quenching DEVD sequence, the free dye excites at 488 nm and emits at 515-530 nm. As a result of a novel and proprietary Lysis Buffer System, the APO 3/7 HTS Assay is a homogenous platform that can be utilized for high throughput fluorescence plate reader applications. The reagent is directly added to the samples thus eliminating any wash steps.



*In this figure, Jurkat cells were stimulated with various concentrations of staurosporine for 3 hours, after which caspase 3/7 activity was analyzed using the APO 3/7 HTS kit.*

### Kit Contents

The APO HTS kit includes the following:

- Part #4004: 1 vial caspase 3/7 reagent (z-DEVD)2 Rodamine 110
- Part #3005: 1 bottle of cell lysis buffer

### Ordering Information

The following APO HTS kits are available:

Catalog No	Quantity	Price (in US dollars)
APO200-2	100 Tests	\$275
APO200-3	500 Tests	\$745
APO200-4	1000 Tests	\$1,395

## References

- Slee, E. A., C. Adrain, and S. J. Martin. 1999. Serial Killers: ordering caspase activation events in apoptosis. *Cell Death and Differ.* 6:1067-1074.
- Walker, N. P., R. V. Talanian, K. D. Brady, L. C. Dang, N. J. Bump, C. R. Ferez, S. Franklin, T. Ghayur, M. C. Hackett and L. D. Hammill. 1994. Crystal Structure of the Cysteine Protease Interleukin-1 $\beta$ -Converting Enzyme: A (p20/p10)<sub>2</sub> Homodimer. *Cell* 78:343-352.
- Wilson, K. P., J. F. Black, J. A. Thomson, E. E. Kim, J. P. Griffith, M. A. Navia, M. A. Murcko, S. P. Chambers, R. A. Aldape, S. A. Raybuck, and D. J. Livingston. 1994. Structure and mechanism of interleukin-1 beta converting enzyme. *Nature* 370: 270-275.
- Rotonda, J., D. W. Nicholson, K. M. Fazil, M. Gallant, Y. Gareau, M. Labelle, E. P. Peterson, D. M. Rasper, R. Ruel, J. P. Vaillancourt, N. A. Thornberry and J.W. Becker. 1996. The three-dimensional structure of apopain/CPP32, a key mediator of apoptosis. *Nature Struct. Biol.* 3(7): 619-625.
- Kumar, S. 1999. Mechanisms mediating caspase activation in cell death. *Cell Death and Differ.* 6: 1060-1066.
- Alnemri, E.S. et al (1996) *Cell* 87:171
- Trends Biochem Sci* 22,388 (1997)

# Carboxyfluorescein Caspase Detection in Live Cells – APO LOGIX™

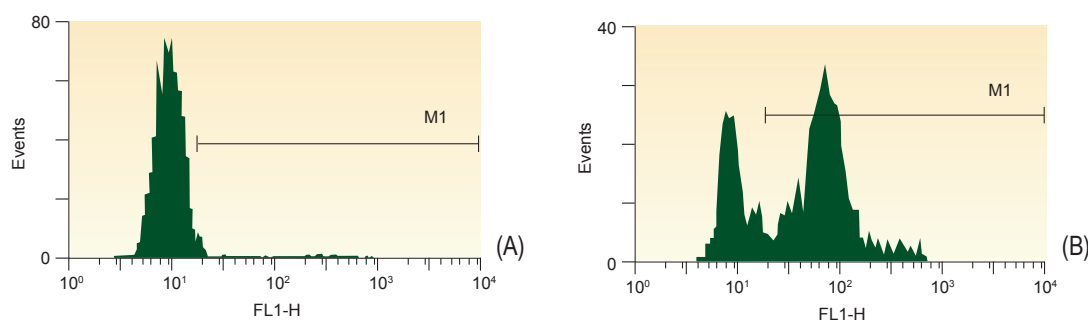
## Key Benefits

The Key Benefits of our the Carboxyfluorescein-Based Caspase Detection Kits are as follows:

- Non-cytotoxic assay arrests further apoptotic activity via caspase inhibition.
- Cell permeability permits direct visualization of cytosolic apoptotic events.
- Apoptotic cell population does not diminish over time.
- Add reagent directly to cells. No special buffer or media needed. No preparation of cell lysates required; simple wash procedure.
- Works in diverse cell lines: human, rodent, Drosophila.
- Can be performed in conjunction with Annexin staining, TUNEL, antibody staining, or with other APO LOGIX™ reagents on the same population of cells.
- Permits high throughput screening. Protocol can be adapted for ex vivo as well as in situ experiments.
- Works with fluorescence microscopy, 96-well fluorescence plate readers, or flow cytometry
- Yields both quantitative and qualitative results. Gives strong signal with little background noise.
- Mark activity across the range of caspase proteins. Poly-caspase and caspase-specific assays to target caspases 1, 2, 3, 6, 8, 9, or 10 are available.

## Assay Principle

APO LOGIX™ Carboxyfluorescein Caspase Detection Kits label active caspases in living cells undergoing apoptosis. Cell Technology's probes utilize carboxyfluorescein (FAM)-labeled peptide fluoromethyl ketone (FMK) caspase inhibitors (FAM-peptide-FMK). These FAM-peptide-FMK compounds are both cell permeable and non-cytotoxic during the course of the assay and thus allow the detection of active caspases in living cell systems.



Above: Jurkat cells treated with DMSO (A) or camptothecin (B). Cells were labeled with FAM-VAD-FMK for 1 hour. Caspase activity was detected using flow cytometry.

## Kit Contents

The Carboxyfluorescein-based Caspase Detection Kit includes the following:

- Part #8001: Lyophilized FAM labeled peptide inhibitor
- Part #3028: 10X Wash Buffer
- Part #3027: 10X Fixative
- Part #4013: Propidium Iodide

## Ordering Information

The following Carboxyfluorescein-Based Caspase Detection Kits are available:

Product	Catalog No.	Quantity	Price (in US dollars)
FAM-VAD-FMK Poly caspase detection kit	FAM100-1	25 Tests	\$155
	FAM100-2	100 Tests	\$445
FAM-DEVD-FMK Caspase 3 detection kit	FAM200-1	25 Tests	\$155
	FAM200-2	100 Tests	\$445
FAM-DEVD-FMK Caspase 8 detection kit	FAM300-1	25 Tests	\$155
	FAM300-2	100 Tests	\$445
FAM-LEHD-FMK Caspase 9 detection kit	FAM400-1	25 Tests	\$155
	FAM400-2	100 Tests	\$445
FAM-VEID-FMK Caspase 6 detection kit	FAM500-1	25 Tests	\$225
	FAM500-2	100 Tests	\$525
FAM-YVAD-FMK Caspase 1 detection kit	FAM600-1	25 Tests	\$155
	FAM600-2	100 Tests	\$445
FAM-VDVAD-FMK Caspase 2 detection kit	FAM700-1	25 Tests	\$225
	FAM700-2	100 Tests	\$525
FAM-AEVD-FMK Caspase 10 detection kit	FAM800-1	25 Tests	\$225
	FAM800-2	100 Tests	\$525

## References

- Slee, E. A., C. Adrain, and S. J. Martin. 1999. Serial Killers: ordering caspase activation events in apoptosis. *Cell Death and Differ.* 6:1067-1074.
- Walker, N. P., R. V. Talanian, K. D. Brady, L. C. Dang, N. J. Bump, C. R. Ferenz, S. Franklin, T. Ghayur, M. C. Hackett and L. D. Hammill. 1994. Crystal Structure of the Cysteine Protease Interleukin-1 $\beta$ -Converting Enzyme: A (p20/p10)<sub>2</sub> Homodimer. *Cell* 78:343-352.
- Wilson, K. P., J. F. Black, J. A. Thomson, E. E. Kim, J. P. Griffith, M. A. Navia, M. A. Murcko, S. P. Chambers, R. A. Aldape, S. A. Raybuck, and D. J. Livingston. 1994. Structure and mechanism of interleukin-1 beta converting enzyme. *Nature* 370: 270-275.
- Rotonda, J., D. W. Nicholson, K. M. Fazil, M. Gallant, Y. Gareau, M. Labelle, E. P. Peterson, D. M. Rasper, R. Ruel, J. P. Vaillancourt, N. A. Thornberry and J. W. Becker. 1996. The three-dimensional structure of apopain/CPP32, a key mediator of apoptosis. *Nature Struct. Biol.* 3(7): 619-625.
- Kumar, S. 1999. Mechanisms mediating caspase activation in cell death. *Cell Death and Differ.* 6: 1060-1066.
- Thornberry, N. A., T. A. Rano, E. P. Peterson, D. M. Rasper, T. Timkey, M. Garcia-Calvo, V. M. Houtzager, P. A. Nordstrom, S. Roy, J. P. Vaillancourt, K. T. Chapman and D. W. Nicholson. 1997. A combinatorial approach defines specificities of members of the caspase family and granzyme B. Functional relationships established for key mediators of apoptosis. *J. Biol. Chem.* 272(29): 17907-17911.
- Amstad, P.A., G.L. Johnson, B.W. Lee and S. Dhawan. 2000. An in situ marker for the detection of activated caspases. *Biotechnology Laboratory* 18: 52-56.
- Bedner, E., P. Smolewski, P.A. Amstad and Z. Darzynkiewicz. 2000. Activation of caspases measured in situ by binding of fluorochrome-labeled inhibitors of caspases (FLICA): correlation with DNA fragmentation. *Exp. Cell Research* 259: 308-313.
- Smolewski, P., E. Bedner, L. Du, T.-C. Hsieh, J. Wu, J. D. Phelps and Z. Darzynkiewicz. 2001. Detection of caspase activation by fluorochrome-labeled inhibitors: multiparameter analysis by laser scanning cytometry. *Cytometry* 44: 73-82.
- Ekert, P. G., J. Silke and D. L. Vaux. 1999. Caspase inhibitors. *Cell Death and Differ.* 6:1081-1086.
- Garcia-Calvo, M., E. Peterson, B. Leiting, R. Ruel, D. Nicholson and N. Thornberry. 1998. Inhibition of human caspases by peptide-based and macromolecular inhibitors. *J. Biol. Chem.* 273: 32608-32613.
- Hirata, H., A. Takahashi, S. Kobayashi, S. Yonehara, H. Sawai, T. Okazaki, K. Yamamoto and M. Sasada. 1998. Caspases are activated in a branched protease cascade and control distinct downstream processes in Fas-induced apoptosis. *J. Exp. Med.*

## Sulforhodamine-Based Caspase Detection Kits

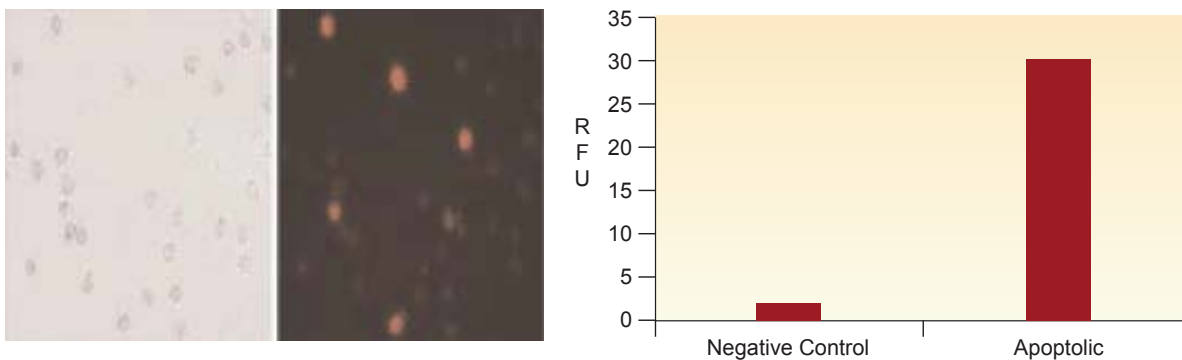
### Key Benefits

The Key Benefits of our Sulforhodamine-Based Caspase Detection Kits are as follows:

- Non-cytotoxic assay arrests further apoptotic activity via caspase inhibition.
- Cell permeability permits direct visualization of cytosolic apoptotic events.
- Apoptotic cell population does not diminish over time.
- Add reagent directly to cells. No special buffer or media needed. No preparation of cell lysates required; simple wash procedure.
- Works in diverse cell lines: human, rodent, *Drosophila*.
- Can be performed in conjunction with Annexin staining, TUNEL, antibody staining, or with other APO LOGIX™ reagents on the same population of cells.
- Permits high throughput screening. Protocol can be adapted for ex vivo as well as in situ experiments.
- Works with fluorescence microscopy, 96-well fluorescence plate readers, or flow cytometry.
- Yields both quantitative and qualitative results. Gives strong signal with little background noise.

### Assay Principle

APO LOGIX™ SR kits contain a generic sulforhodamine labeled caspase inhibitor (sulforhodamine-peptide-fluoromethyl ketone). This reagent is cell permeable and is used on whole cells to detect apoptosis. Apoptotic cells are detected by a fluorescence plate reader or fluorescence microscope using an excitation source at 550 nm and measuring emission at 595 nm. The assay takes about one hour to complete.



*Above: Jurkat cells stimulated with staurosporine for 2 hours and then labeled with SR-VAD-FMK. Left side: 30X phase contrast. Right side: 30X fluorescence microscope. Excitation: 550nm emission > 580nm.*

### Kit Contents

The sulforhodamine-based caspase detection kit includes the following:

- Lyophilized SR-VAD-FMK
- 10X Wash Buffer
- 10X fixative

## Ordering Information

The following Sulforhodamine-Based Caspase Detection kits are available:

Product	Catalog No.	Quantity	Price (in US dollars)
SR-VAD-FMK Poly caspase detection kit	SR100-1	25 Tests	\$225
	SR100-2	100 Tests	\$575

## References

- Slee, E. A., C. Adrain, and S. J. Martin. 1999. Serial Killers: ordering caspase activation events in apoptosis. *Cell Death and Differ.* 6:1067-1074.
- Walker, N. P., R. V. Talanian, K. D. Brady, L. C. Dang, N. J. Bump, C. R. Ferenz, S. Franklin, T. Ghayur, M. C. Hackett and L. D. Hammill. 1994. Crystal Structure of the Cysteine Protease Interleukin-1 $\beta$ -Converting Enzyme: A (p20/p10)<sub>2</sub> Homodimer. *Cell* 78:343-352.
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- Bedner, E., P. Smolewski, P.A. Amstad and Z. Darzynkiewicz. 2000. Activation of caspases measured in situ by binding of luorochrome-labeled inhibitors of caspases (FLICA): correlation with DNA fragmentation. *Exp. Cell Research* 259: 308-313.
- Smolewski, P., E. Bedner, L. Du, T.-C. Hsieh, J. Wu, J. D. Phelps and Z. Darzynkiewicz. 2001. Detection of caspase activation by fluorochrome-labeled inhibitors: multiparameter analysis by laser scanning cytometry. *Cytometry* 44: 73-82.
- Ekert, P. G., J. Silke and D. L. Vaux. 1999. Caspase inhibitors. *Cell Death and Differ.* 6:1081-1086.
- Garcia-Calvo, M., E. Peterson, B. Leiting, R. Ruel, D. Nicholson and N. Thornberry. 1998. Inhibition of human caspases by peptidebased and macromolecular inhibitors. *J. Biol. Chem.* 273: 32608-32613.
- Hirata, H., A. Takahashi, S. Kobayashi, S. Yonehara, H. Sawai, T. Okazaki, K. Yamamoto and M. Sasada. 1998. Caspases are activated in a branched protease cascade and control distinct downstream processes in Fas-induced apoptosis. *J. Exp. Med.* 187: 587-600.

## Dual Sensor Caspase & MMP Detection - MitoCasp™

### Key Benefits

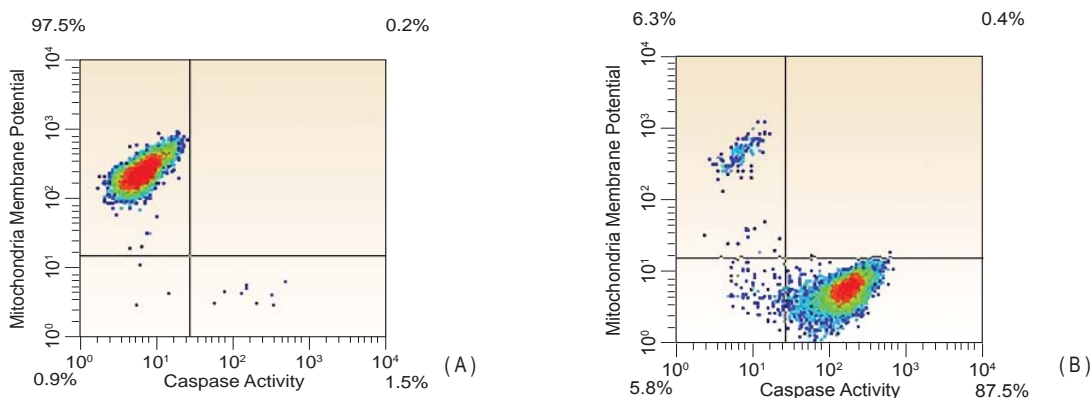
The Key Benefits of our Dual Sensor MitoCasp™ kits are as follows:

- Simultaneous detection of mitochondrial membrane potential and caspase activity
- Readout - Flow cytometry, 96-well plate reader, fluorescence microscope
- Reliable: Yields both quantitative and qualitative results; gives a strong positive signal
- The kit can be used in conjunction with other antibodies or stains
- Ease Of Use: No need to make cell lysates or run Western blots
- Cell permeable reagents

### Assay Principle

Caspase enzymes specifically recognize a 4 amino acid sequence (on their substrate) which necessarily includes an aspartic acid residue. This residue is the target for the cleavage reaction, which occurs at the carbonyl end of the aspartic acid residue (6). Caspases can be detected via immunoprecipitation, immuno-blotting techniques using caspase specific antibodies, or by employing fluorogenic substrates which become fluorescent upon cleavage by the caspase. MitoCasp™ uses a novel approach to detect active caspases (7-9). The methodology is based on carboxyfluorescein (FAM) labeled fluoromethyl ketone (FMK)-peptide inhibitors of caspases. These inhibitors are cell permeable and non-cytotoxic. Once inside the cell, the inhibitor binds covalently to the active caspase (10). Cells that contain bound inhibitor can be analyzed by flow cytometry or fluorescence microscopy.

Cell Technology utilizes a cationic dye to visualize mitochondrial membrane potential (15-17). The cationic dye is cell permeable and has a strong fluorescent signal in the red region and exhibits low membrane potential independent (non specific) binding and toxicity. In healthy cells the cationic dye is accumulated by the mitochondria in proportion to the Delta Psi (membrane potential). In most cell lines, accumulation of the cationic dye in the mitochondria results in a higher fluorescence intensity. In apoptotic cells, where the mitochondrial membrane potential is compromised, the cationic dye does not accumulated in the mitochondria and these cells exhibit a lower fluorescence signal. Utilizing these two reagents in combination Caspase activity and mitochondrial membrane potential can be analyzed simultaneously.



In the figures above Jurkat cells were stimulated with Staurosporine for three hours (B) or DMSO (A). The cells were then stained with the MitoCasp kit according to the protocol. The cells were then washed twice and analyzed by flow cytometry: Ex: 488nm Em: FL1 and FL2. In Figure healthy cells show a strong red fluorescence indicating intact mitochondria and no green fluorescence, indicating no active caspases. In Figure B apoptotic cells show a loss of red fluorescence (y axis) indicating loss of mitochondrial membrane potential and positive green fluorescence (x axis) indicating active caspases.

## Kit Contents

The MitoCasp™ kit includes the following:

- Mitochondrial membrane potential cationic dye
- Caspase Detection reagent (Poly Caspase, Caspase 3/7 or Caspase 9)
- 10X Wash Buffer
- 1X Dilution Buffer

## Ordering Information

The following MitoCasp kits are available:

Catalog No.	Quantity	Price (in US dollars)
MITCAP 100-1 (Poly Caspase)	25 Tests	\$225
MITCAP 100-2 (Poly Caspase)	100 Tests	\$645
MITCAP 200-1 (Caspase 3/7)	25Tests	\$225
MITCAP 200-2 (Caspase 3/7)	100Tests	\$645
MITCAP 300-1 (Caspase 8)	25Tests	\$225
MITCAP 300-2 (Caspase 8)	100Tests	\$645
MITCAP 400-1 (Caspase 9)	25Tests	\$225
MITCAP 400-2 (Caspase 9)	100Tests	\$645
MITCAP 600-1 (Caspase 1)	25Tests	\$225
MITCAP 600-2 (Caspase 1)	100Tests	\$645

## References

- Slee, E. A., C. Adrain, and S. J. Martin. 1999. Serial Killers: ordering caspase activation events in apoptosis. *Cell Death and Differ.* 6:1067-1074.
- Walker, N. P., R. V. Talanian, K. D. Brady, L. C. Dang, N. J. Bump, C. R. Ferenz, S. Franklin, T. Ghayur, M. C. Hackett and L. D. Hammill. 1994. Crystal Structure of the Cysteine Protease Interleukin-1 $\beta$ -Converting Enzyme: A (p20/p10)<sub>2</sub> Homodimer. *Cell* 78:343-352.
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- Amstad, P.A., G.L. Johnson, B.W. Lee and S. Dhawan. 2000. An in situ marker for the detection of activated caspases. *Biotechnology Laboratory* 18: 52-56.
- Bedner, E., P. Smolewski, P.A. Amstad and Z. Darzynkiewicz. 2000. Activation of caspases measured in situ by binding or fluorochrome-labeled inhibitors of caspases (FLICA): correlation with DNA fragmentation. *Exp. Cell Research* 259: 308-313.
- Smolewski, P., E. Bedner, L. Du, T.-C. Hsieh, J. Wu, J. D. Phelps and Z. Darzynkiewicz. 2001. Detection of caspase activation by fluorochrome-labeled inhibitors: multiparameter analysis by laser scanning cytometry. *Cytometry* 44: 73-82.
- Ekert, P. G., J. Silke and D. L. Vaux. 1999. Caspase inhibitors. *Cell Death and Differ.* 6:1081-1086.
- Desagher, S., Osen-Sand, A., Nichols, A., Eskes, R., Montessuit, S., Lauper, S., Maundrell, K., Antonsson, B., and Martinou, J.C. Bidinduced conformational change of Bax is responsible for mitochondrial cytochrome c release during apoptosis. *J. Cell Biol.* 144 (5): 891-901 (1999).
- Narita, M., Shimizu, S., Ito, T., Chittenden, T., Lutz, R. J., Matsuda, H., and Tsujimoto, Y. Bax interacts with the permeability transition pore to induce permeability transition and cytochrome c release in isolated mitochondria. *Proc. Natl. Acad. Sci. USA* 95: 14681-14686 (1998).
- Basanez, G., Nechushtan, A., Drozhinin, O., Chanturiya, A., Choe, E., Tutt, S., Wood, K. A., Hsu, Y. T., Zimmerberg, J., and Youle, R. J. Bax, but not Bcl-XL decreases the lifetime of planar phospholipid bilayer membranes at subnanomolar concentrations. *Proc. Natl. Acad. Sci. USA* 96: 5492-5497 (1999).

Luo, X., Budihardjo, I., Zou, H., Slaughter, C., and Wang, X. Bid, a Bcl-2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. *Cell* 94: 481-490 (1998).

Ehrenberg B, Montana V, Wei MD, Wuskell JP, Loew LM. Membrane potential can be determined in individual cells from the nernstian distribution of cationic dyes. *Biophys J.* 1988 May;53(5):785-94.

Farkas DL, Wei MD, Febroriello P, Carson JH, Loew LM. Simultaneous imaging of cell and mitochondrial membrane potentials. : *Biophys J.* 1989 Dec;56(6):1053-69. Erratum in: *Biophys J* 1990 Mar;57(3):following 684.

Russell C. Scaduto, Jr. and Lee W. Grotyohann. Measurement of mitochondrial membrane potential using fluorescent rhodamine derivatives. *Biophys J.* 1999 Jan;76(1 Pt 1):469-77.

Rajagopal A, Pant AC, Simon SM, Chen Y. In vivo analysis of human multidrug resistance protein 1 (MRP1) activity using transient expression of fluorescently tagged MRP1. *Cancer Res.* 2002 Jan 15; 62 (2):391-6.

## Antibody-Specific Active Caspase 3 Detection – Apo Active 3™ FITC

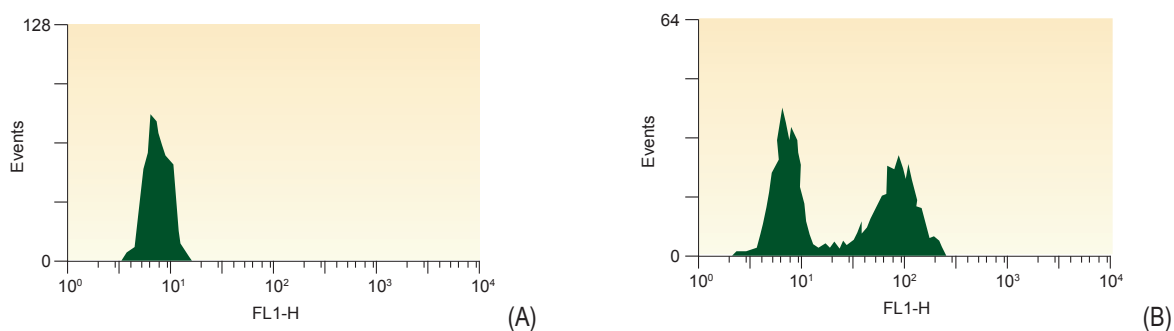
### Key Benefits

The Key Benefits of our APO ACTIVE 3™ Detection Kits are as follows:

- Highly specific for active human and murine caspase 3; other assays require the utilization of peptide based (DEVD) reagents that tend to cross-react with caspase 7 and other caspases.
- Works with a fluorescence microscope, 96-well plate reader, or flow cytometer.
- Yields both quantitative and qualitative results. Gives a strong positive signal.
- Can be used in conjunction with other antibodies or stains.
- No need to make cell lysates or run Western blots. Cells can be fixed and analyzed later

### Assay Principle

Cell Technology's APO-ACTIVE CASPASE 3 Kit utilizes a rabbit affinity purified polyclonal antibody raised against amino acid 163-175 of murine caspase 3<sup>(9)</sup>. This neo epitope is present on the p18 subunit of cleaved caspase 3<sup>(9)</sup>. Cells undergoing apoptosis are fixed and permeabilized prior to the addition of the antibody. A secondary FITC labeled goat anti rabbit antibody is used to visualize the bound rabbit anti caspase 3 polyclonal.



Above: Jurkat Cells were stimulated with staurosporine (B) or DMSO (A) for 2 hours. The cells were washed and fixed for 15 minutes. The cells were then permeabilized with saponin and stained with Rabbit anti active caspase 3 antibody for 1 hour. The samples were then stained with FITC labeled Goat anti Rabbit antibody.

### Kit Contents

The APO ACTIVE 3 Detection Kit includes the following:

- 1 vial of Rabbit anti cleaved caspase 3 affinity purified polyclonal antibody
- 1 vial of FITC labeled Goat anti Rabbit affinity purified polyclonal antibody
- 1 vial of 10X fixative.

## Ordering Information

The following APO ACTIVE 3™ Detection Kits are available:

Catalog No.	Quantity	Price (in US dollars)
FAB200-1	25 Tests	\$155
FAB200-2	100 Tests	\$445

## References

- Slee, E. A., C. Adrain, and S. J. Martin. 1999. Serial Killers: ordering caspase activation events in apoptosis. *Cell Death and Differ.* 6:1067-1074.
- Walker, N. P., R. V. Talanian, K. D. Brady, L. C. Dang, N. J. Bump, C. R. Ferez, S. Franklin, T. Ghayur, M. C. Hackett and L. D. Hammill. 1994. Crystal Structure of the Cysteine Protease Interleukin-1 $\beta$ -Converting Enzyme: A (p20/p10)<sub>2</sub> Homodimer. *Cell* 78:343-352.
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- Rotonda, J., D. W. Nicholson, K. M. Fazil, M. Gallant, Y. Gareau, M. Labelle, E. P. Peterson, D. M. Rasper, R. Ruel, J. P. Vaillancourt, N. A. Thornberry and J. W. Becker. 1996. The three-dimensional structure of apopain/CPP32, a key mediator of apoptosis. *Nature Struct. Biol.* 3(7): 619-625.
- Kumar, S. 1999. Mechanisms mediating caspase activation in cell death. *Cell Death and Differ.* 6: 1060-1066.
- Alnemri, E.S. et al (1996) *Cell* 87:171
- Trends Biochem Sci* 22,388 (1997)
- Nature* 376, 37 (1995).
- Srinivasan A, et al *Cell Death and Differentiation* (1998) 5: 1004-1016

## Phycoerythrin Antibody-Based Caspase 3 Detection – APO ACTIVE 3™ PE

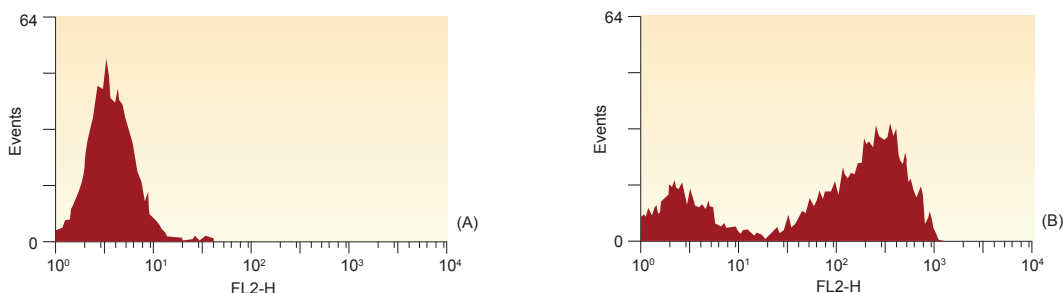
### Key Benefits

The Key Benefits of our APO ACTIVE 3™ PE Detection Kits are as follows:

- Highly specific for active human and murine caspase 3. Other assays require the utilization of peptide based (DEVD) reagents that tend to cross-react with caspase 7 and other caspases.
- Works with a flow cytometer, fluorescence microscope, or 96-well plate reader.
- Yields both quantitative and qualitative results; gives a strong positive signal.
- Can be used in conjunction with other antibodies or stains.
- No need to make cell lysates or run Western blots. Cells can be fixed and analyzed later.
- Works with human, mouse and rat cell lines.

### Assay Principle

Cell Technology's APO-ACTIVE 3™ PE Kit utilizes a rabbit affinity purified polyclonal antibody raised against amino acid 163-175 of murine caspase 3<sup>(9)</sup>. This neo epitope is present on the p18 subunit of cleaved caspase 3<sup>(9)</sup>. Cells undergoing apoptosis are fixed and permeabilized prior to the addition of the antibody. A secondary PE labeled goat anti rabbit antibody is used to visualize the bound rabbit anti caspase 3 polyclonal. PE labeling allows for use with a variety of applications.



Above: Jurkat Cells were stimulated with staurosporine (B) or DMSO (A) for 2 hours. The cells were washed and fixed for 15 minutes. The cells were then permeabilized with saponin and stained with Rabbit anti active caspase 3 antibody for 1 hour. The samples were then stained with PE labeled Goat anti Rabbit antibody.

### Kit Contents

The APO ACTIVE 3™ PE Detection Kits include the following:

- 1 vial of Rabbit anti cleaved caspase 3 affinity purified polyclonal antibody
- 1 vial of PE labeled Goat anti Rabbit affinity purified polyclonal antibody
- 1 vial of 10X fixative

## Ordering Information

The following APO ACTIVE 3™ PE Antibody-specific Active Caspase 3 Detection Kits are available:

Catalog No.	Quantity	Price (in US dollars)
PAB200-1	25 Tests	\$155
PAB200-2	100 Tests	\$445

## References

- Slee, E. A., C. Adrain, and S. J. Martin. 1999. Serial Killers: ordering caspase activation events in apoptosis. *Cell Death and Differ.* 6:1067-1074.
- Walker, N. P., R. V. Talanian, K. D. Brady, L. C. Dang, N. J. Bump, C. R. Ferez, S. Franklin, T. Ghayur, M. C. Hackett and L. D. Hammill. 1994. Crystal Structure of the Cysteine Protease Interleukin-1 $\beta$ -Converting Enzyme: A (p20/p10)<sub>2</sub> Homodimer. *Cell* 78:343-352.
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- Alnemri, E.S. et al (1996) *Cell* 87:171
- Trends Biochem Sci* 22,388 (1997)
- Nature* 376, 37 (1995).

# Apoptosis Induced ssDNA Damage Assay - ApossDNA™

## Key Benefits

The Key Benefits of our Apoptosis Induced single-stranded DNA Damage Assay Kits are as follows:

- Much more robust as compared to TUNEL assays; no false positive signals
- Readout - Flow cytometry, 96-well plate reader, fluorescence microscope
- Reliable: Yields both quantitative and qualitative results; gives a strong positive signal.
- Ease Of Use: Results in less than 60 minutes
- No need to run DNA ladder assays to detect DNA damage

## Introduction

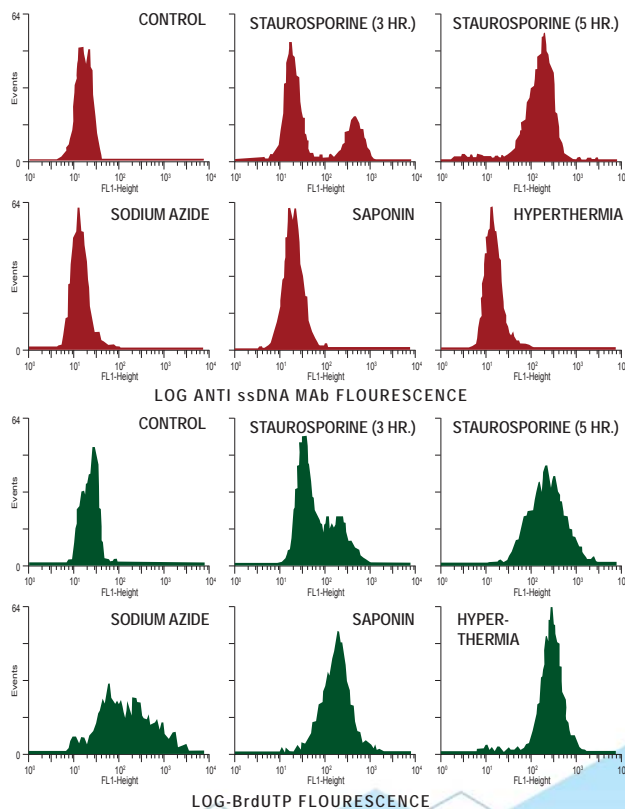
A widely used cytochemical technique for evaluation of DNA damage associated with apoptosis is the terminal deoxynucleotidyl transferase-mediated in situ end labeling or TUNEL assay. However, the TUNEL assay has its drawbacks in that false positive staining makes the assay unreliable as a marker for apoptosis (see Figure A) (1-5). A more universal and specific marker for apoptosis is the morphological changes in nuclei that reflect chromatin condensation in to compact masses (6-7).

Further biochemical and cytochemical studies have demonstrated the increased susceptibility of apoptotic DNA to thermal denaturation. Analysis of nuclei by scanning calorimetry to detect thermal induced DNA denaturation and analysis of DNA fragmentation by electrophoresis have shown that intact apoptotic DNA is susceptible to denaturation at lower temperatures than that of non-apoptotic cells (8).

## Assay Principle

Cell Technology introduces Apo ssDNA™, an antibody based assay to detect DNA damage (single stranded DNA: ssDNA) in Apoptotic Cells.

The assay utilizes an antibody generated against ssDNA. This antibody recognizes large stretches of heat-denatured ssDNA only in apoptotic cells (9-12).



*Figure A: MAb to ssDNA staining of apoptotic but not of necrotic cells. Fluorescence distributions of MDA-468 cells heated in formamide and stained with MAb F7-26 were generated on a flow cytometer. Apoptosis was induced by staurosporine and necrosis was induced by sodium azide, saponin, or hyperthermia. Note that apoptotic cells are intensely stained with the MAb, whereas the fluorescence profiles of necrotic and control cells are similar.*

*Figure B: TUNEL staining of apoptotic and necrotic cells. Fluorescence distributions of MDA-468 cells stained with TUNEL were generated on a flow cytometer. Apoptosis was induced by staurosporine and necrosis was induced by sodium azide, saponin, or hyperthermia. Note that cells at early stage of apoptosis (staurosporine 3 hr) are weakly stained, whereas late apoptotic cells (staurosporine 5 hr) and necrotic cells are intensely stained by TUNEL (12).*

## Kit Contents

The Apoptosis Induced single-stranded DNA Damage Assay Kits include the following:

- Mouse anti single stranded DNA -70°C aliquot
- Anti mouse IgM FITC labeled -20°C
- Block Buffer 2-8°C
- 10 X Wash Buffer 2-8°C

## Ordering Information

The following Apoptosis Induced single-stranded DNA Damage Assay Kits are available:

Catalog No.	Quantity	Price (in US dollars)
ApoDNA-1	25 Tests	\$225
ApoDNA-2	100 Tests	\$745

## References

Charriat–Marlangue C, Ben-Ari J (1995) A cautionary note on the use of TUNEL to determine apoptosis. *NeuroReport* 7:61–64

Grasl–Kraipp B, Ruttkau–Nedecky B, Koudelka H, Bukowska K, Bursch W, Schulte–Hermann R (1995) In situ detection of fragmented DNA (TUNEL) fails to discriminate among apoptosis, necrosis and autolytic cell death: a cautionary note. *Hepatology* 21:1465–146

Didenko VV, Hornsby PJ (1996) Presence of double-stranded DNA breaks with single-base 39 overhangs in cells undergoing apoptosis but not necrosis. *J Cell Biol* 135:1369–1376

Ohno M, Takemura G, Ohno A, Misao J, Hayakawa Y, Minatogu-chi S, Fujiwara T, Fujiwara H, (1998) “Apoptotic” myocytes infarct area in rabbit hearts may be oncotic myocytes with DNA fragmentation: analysis by immunogold electron microscopy combined with in situ nick end-labeling. *Circulation* 98:1422–1430.

Stadelmann C, Bruck W, Bancher C, Jellinger K, Lassmann H (1998) Alzheimer disease: DNA fragmentation indicates in-creased neuronal vulnerability, but not apoptosis. *Neuropathol Exp Neurol* 57:456–464

Willingham MC (1999) Cytochemical methods for the detection of apoptosis. *J Histochem Cytochem* 47:1101–1109

Zamzani N, Kroemer J (1999) Condensed matter in cell death. *Nature* 401:127–128.

Allera C, Lazzarini G, Patrone E, Alberti I, Barboro P, Sanna P, Melchiori, A, Parodi S, Balbi C (1997) Condensation of chromatin in apoptotic thymocytes shows a specific structural change. *J. Biol Chem* 272:10817–10822.

Frankfurt OS (1990) Decreased DNA stability in cells treated with alkylating agents. *Exp Cell Res* 191:181–185.

Frankfurt OS, Robb JA, Sugarbaker EV, Villa L (1996) Monoclonal antibody to single-stranded DNA is a specific and sensitive cellular marker of apoptosis. *Exp Cell Res* 226:387–397.

Frankfurt OS (1994) Detection of apoptosis in leukemic and breast cancer cells with monoclonal antibody to single-stranded DNA. *Anticancer Res* 14:1861–1870.

Oskar S. Frankfurt and Awtar Krishan (2001). Identification of Apoptotic Cells by Formamide-induced DNA Denaturation in Condensed Chromatin. *The Journal of Histochemistry & Cytochemistry* Volume 49(3): 369–378, 2001

# Dual Sensor Antibody Based ssDNA Damage & Caspase 3 Assay - DNACASPTM

## Key Benefits

The Key Benefits of our Dual Sensor Antibody Based ssDNA Damage & Caspase 3 Assay Kits are as follows:

- Much more Reliable as compared to TUNEL assays - No False positive signals
- Multiple parameter detection of DNA damage and caspase 3 activity simultaneously
- Readout - Flow cytometry, Fluorescence 96 well plate reader, Fluorescence microscope
- Ease Of Use: Dual parameter results to confirm apoptosis in cells
- Compatible with human, mouse, rat, bovine, porcine species

## Introduction

### 1. Antibody to Single Stranded DNA

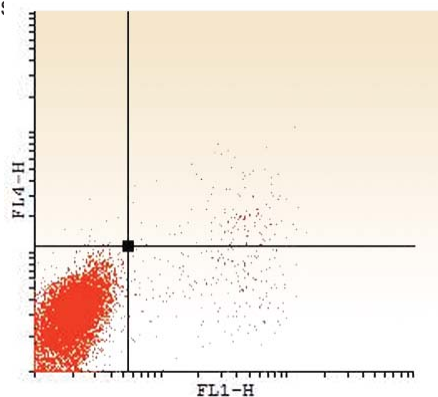
A widely used cytochemical technique for evaluation of DNA damage associated with apoptosis is the terminal deoxynucleotidyl transferase-mediated in situ end labeling or TUNEL assay. However the TUNEL assay has its drawbacks in that false positive staining makes the assay unreliable as a marker for apoptosis 1-5. A more universal and specific marker for detecting apoptosis associated DNA damage is to measure the morphological changes in nuclei that reflect chromatin condensation into compact masses. 6-7. Further biochemical and cytochemical studies have demonstrated the increased susceptibility of apoptotic DNA to thermal denaturation. Analysis of nuclei by scanning calorimetry to detect thermal induced DNA denaturation and analysis of DNA fragmentation by electrophoresis have shown that intact apoptotic DNA is susceptible to denaturation at lower temperatures than that of non-apoptotic cells 8.

### 2. Antibody to Active Caspase 3

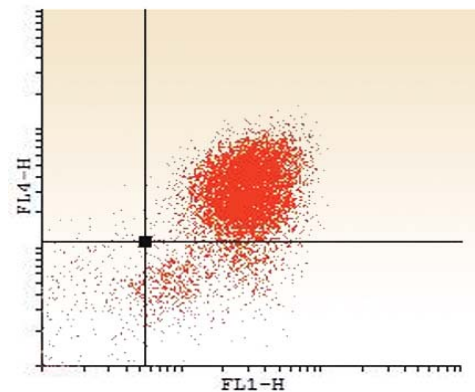
Apoptosis is an evolutionarily conserved form of cell suicide, which follows a specialized cellular process. The central component of this process is a cascade of proteolytic enzymes called caspases. These enzymes participate in a series of reactions that are triggered in response to pro-apoptotic signals and result in cleavage of protein substrates, causing the disassembly of the cell 13. Caspases have been identified in organisms ranging from *C. elegans* to humans. The mammalian caspases play distinct roles in apoptosis and inflammation. In apoptosis, caspases are responsible for proteolytic cleavages that lead to cell disassembly (effector caspases), and are involved in upstream regulatory events (initiator caspases).

## Assay Principle

Cell Technology introduces a dual parameter antibody based assay to detect DNA damage (heat denatured single stranded DNA: ssDNA)<sup>9-12</sup> and active caspase 3 in apoptotic cells. The assay utilizes a monoclonal antibody generated against ssDNA and a primary rabbit affinity purified polyclonal antibody raised against amino acid 163-175 of murine caspase 3<sup>12</sup>. This neo epitope is present on the p18:



*Fig (A): Negative control: Jurkat cells treated with DMSO for 3 hours and treated with 1 mM staurosporine for 3 hours. The cells were stained with Fluoro ssDNA Caspase 3 kit as described in the ssDNA Caspase 3 Kit as described in the protocol. Key: FL 1-H = Anti active ssDNA stain (DNA damage), FL 4-H = anti*



*Fig (B): Positive Control: Jurkat cells treated for 3 hours. The cells were stained with Fluoro ssDNA Caspase 3 kit as described in the protocol. Key FL 1-H = Anti*

## Kit Contents

The Dual Sensor Antibody Based ssDNA Damage & Caspase 3 Assay Kits include the following:

- Mouse anti single stranded DNA -70°C aliquot
- Rabbit anti active Caspase 3 2-8°C
- Goat Anti mouse FITC labeled 2-8°C
- Goat Anti Rabbit APC 2-8°C
- Fixative 2-8°C
- 10 X Wash Buffer -20°C
- Block Buffer -20°C
- 1X Denaturing Buffer -20°C

## Ordering Information

The following Dual Sensor Antibody Based ssDNA Damage & Caspase 3 Assay Kits are available:

Catalog No.	Quantity	Price (in US dollars)
DNACASP3-1	25 Tests	\$325
DNACASP3-2	50 Tests	\$625

## References

Charriat-Marlangue C, Ben-Ari J (1995) A cautionary note on the use of TUNEL to determine apoptosis. *NeuroReport* 7:61–64

Grasl-Kraipp B, Ruttkau-Nedecky B, Koudelka H, Bukowska K, Bursch W, Schulte-Hermann R (1995) In situ detection of fragmented DNA (TUNEL) fails to discriminate among apoptosis, necrosis and autolytic cell death: a cautionary note. *Hepatology* 21:1465–146

Didenko VV, Hornsby PJ (1996) Presence of double-stranded DNA breaks with single-base 3' overhangs in cells undergoing apoptosis but not necrosis. *J Cell Biol* 135:1369–1376

Ohno M, Takemura G, Ohno A, Misao J, Hayakawa Y, Minatogu-chi S, Fujiwara T, Fujiwara H, (1998) "Apoptotic" myocytes infarct area in rabbit hearts may be oncotic myocytes with DNA fragmentation: analysis by immunogold electron microscopy combined with in situ nick end-labeling. *Circulation* 98:1422–1430.

# Section 3

## **Section 3: Apoptosis / Mitochondria Membrane Potential Detection**

## Mitochondrial Membrane Potential Kit – JC 1

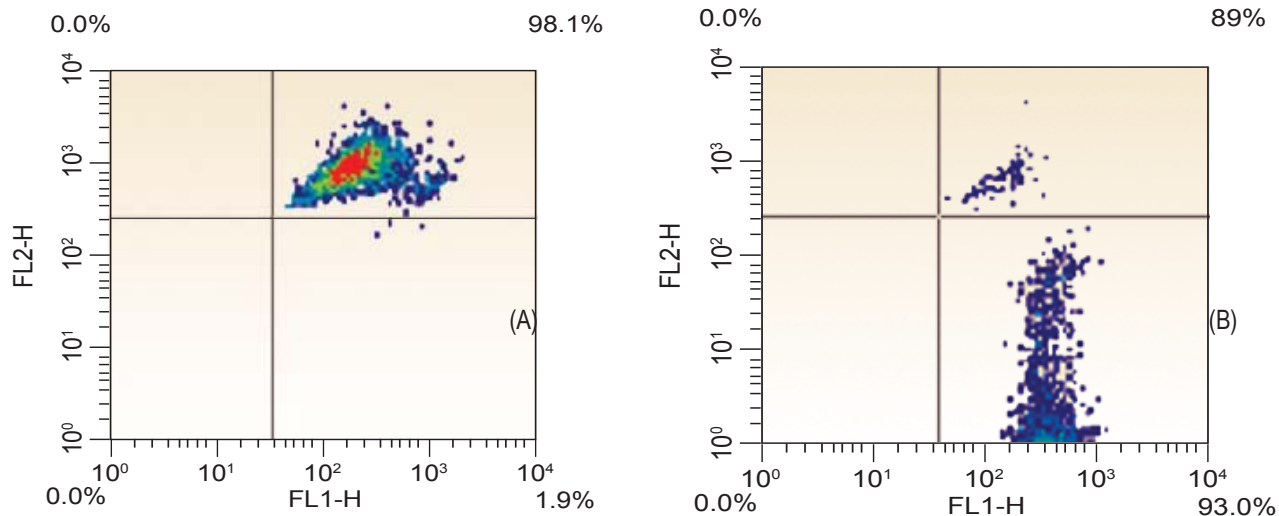
### Key Benefits

The Key Benefits of our the Mitochondrial Membrane Potential Kit-JC1 are as follows:

- Cell permeability allows direct measurement of apoptosis and mitochondrial potential in live cells.
- Cell can be analyzed by flow cytometry, 96-well plate reader or fluorescence microscope.
- Incubate for 15 minutes, wash and measure.
- Add this reagent directly to live cells in your media of choice.

### Assay Principle

Detection of the mitochondrial permeability transition event provides an early indication of the initiation of cellular apoptosis. This process is typically defined as a collapse in the electrochemical gradient across the mitochondrial membrane, as measured by the change in the membrane potential ( $\Psi$ ). Loss of mitochondrial  $\Psi$  is indicative of apoptosis and can be detected by a unique fluorescent cationic dye, 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzamidazolocarboyanin iodide, commonly known as JC-1. This dye has been incorporated into the user-friendly kit for the simple and reproducible detection of the membrane potential ( $\Psi$ ) event in apoptotic cells. The kit has been formatted for use on flow cytometers fluorometric plate readers.



Above: Jurkat cells were cultured with DMSO (A) or staurosporine (B) for 2 hours. Cell were then stained with JC-1 Mitochondrial Membrane Potential Detection Kit for 15 minutes and analyzed by flow cytometry.

### Kit Contents

The Mitochondrial Membrane Potential Kit-JC1 kit includes the following:

- Part #4001: Lyophilized JC-1 Dye
- Part #3002: 10X Wash Buffer

## Ordering Information

The following Mitochondrial Membrane Potential, APO LOGIX™ JC-1 kit is available:

Catalog No.	Quantity	Price (in US dollars)
JC100	100 Tests	\$275

## References

- Desagher, S., Osen-Sand, A., Nichols, A., Eskes, R., Montessuit, S., Lauper, S., Maundrell, K., Antonsson, B., and Martinou, J.C. Bid-induced conformational change of Bax is responsible for mitochondrial cytochrome c release during apoptosis. *J. Cell Biol.* 144 (5):891-901 (1999).
- Narita, M., Shimizu, S., Ito, T., Chittenden, T., Lutz, R. J., Matsuda, H., and Tsujimoto, Y. Bax interacts with the permeability transition pore to induce permeability transition and cytochrome c release in isolated mitochondria. *Proc. Natl. Acad. Sci. USA* 95: 14681- 14686 (1998).
- Basanez, G., Nechushtan, A., Drozhinin, O., Chanturiya, A., Choe, E., Tutt, S., Wood, K. A., Hsu, Y. T., Zimmerberg, J., and Youle, R. J. Bax, but not Bcl-XL decreases the lifetime of planar phospholipid bilayer membranes at subnanomolar concentrations. *Proc. Natl. Acad. Sci. USA* 96: 5492-5497 (1999).
- Luo, X., Budihardjo, I., Zou, H., Slaughter, C., and Wang, X. Bid, a Bcl-2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. *Cell* 94: 481-490 (1998).
- Smiley, S. T., Reers, M., Mottola-Hartshorn, C., Lin, M., Chen, A., Smith, T. W., Steele, G.D., and Chen, L. B. Intracellular heterogeneity in mitochondrial membrane potentials revealed by a J-aggregate forming lipophilic cation JC-1. *Proc. Natl. Acad. Sci. USA* 88: 3671- 3675 (1991).
- Cossarizza, A., Baccarani-Contri, M., Kalashnikova, G., and Franceschi, C. A new method for the cytofluorimetric analysis of mitochondrial membrane potential using the J-aggregate forming lipophilic cation 5,5',6,6'-tetrachloro-1,1',3,3'- tetraethylbenzimidazolylcarbocyanine iodide (JC-1). *Biochem. Biophys. Res. Commun.* 197 (1): 40-45 (1993).
- Reers, M., Smith, T. W., and Chen, L. B. J-aggregate formation of a carbocyanine as a quantitative fluorescent indicator of membrane potential. *Biochemistry* 30: 4480-4486 (1991).
- White, R. J., and Reynolds, I. J. Mitochondrial depolarization in glutamate stimulated neurons: an early signal specific to excitotoxin exposure. *Journal of Neuroscience* 16: 5688-5697 (1996).

## Mitochondrial Membrane Potential Detection – Mito Flo

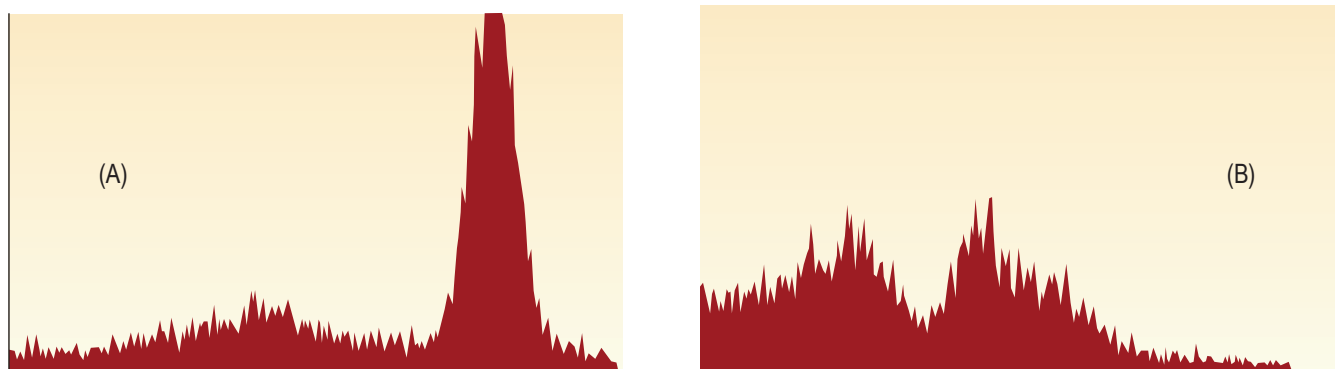
### Key Benefits

The Key Benefits of our Mitochondrial Membrane Potential Detection – Mito Flo Kit are as follows:

- Cell Permeable, Easy One Color Assay for Flow Cytometry
- Can be used with both suspension and monolayer adherent cell lines.
- Compatible with other antibodies or stains. For example fluorescent protein expression vectors.

### Assay Principle

Cell Technology's Mito Flow assay utilizes a derivative of Rhodamine to visualize mitochondrial membrane potential<sup>(5-7)</sup>. The Mito Flow reagent is a cell permeable cationic dye that has a strong fluorescent signal and exhibits low membrane potential independent (non specific) binding and toxicity. In healthy cells the Mito Flow reagent is accumulated by the mitochondria in proportion to the Delta Psi (membrane potential). In most cell lines, accumulation of the Mito Flow reagent in the mitochondria results in a higher fluorescence intensity. In apoptotic cells, where the mitochondrial membrane potential is compromised, the Mito Flow reagent does not get accumulated in the mitochondria and these cells exhibit a lower fluorescence signal.



Above: Jurkat cells were stimulated with Staurosporine (B) or DMSO (A) for 3 hours. Cells were then stained by Mito Flow and analyzed by Flow Cytometry

### Kit Contents

The Mitochondrial Membrane Potential Detection – Mito Flo Kit includes the following:

- Part #4004: Vial of Mito Flow Dye Part
- Part #3004: Bottle of 10X dilution buffer

## Ordering Information

The following Mitochondrial Membrane Potential Detection – Mito Flo Kits are available:

Catalog No.	Quantity	Price (in US dollars)
Flo200-2	100 Tests	\$245
Flo200-3	500 Tests	\$475

## References

Desagher, S., Osen-Sand, A., Nichols, A., Eskes, R., Montessuit, S., Lauper, S., Maundrell, K., Antonsson, B., and Martinou, J.C. Bid-induced conformational change of Bax is responsible for mitochondrial cytochrome c release during apoptosis. *J. Cell Biol.* 144 (5): 891-901 (1999).

Narita, M., Shimizu, S., Ito, T., Chittenden, T., Lutz, R. J., Matsuda, H., and Tsujimoto, Y. Bax interacts with the permeability transition pore to induce permeability transition and cytochrome c release in isolated mitochondria. *Proc. Natl. Acad. Sci. USA* 95: 14681- 14686 (1998).

Basanez, G., Nechushtan, A., Drozhinin, O., Chanturiya, A., Choe, E., Tutt, S., Wood, K. A., Hsu, Y. T., Zimmerberg, J., and Youle, R. J. Bax, but not Bcl-XL decreases the lifetime of planar phospholipid bilayer membranes at subnanomolar concentrations. *Proc. Natl. Acad. Sci. USA* 96: 5492-5497 (1999).

Luo, X., Budihardjo, I., Zou, H., Slaughter, C., and Wang, X. Bid, a Bcl-2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. *Cell* 94: 481-490 (1998).

Ehrenberg B, Montana V, Wei MD, Wuskell JP, Loew LM. Membrane potential can be determined in individual cells from the nernstian distribution of cationic dyes. *Biophys J.* 1988 May; 53(5):785-94.

Farkas DL, Wei MD, Febroriello P, Carson JH, Loew LM. Simultaneous imaging of cell and mitochondrial membrane potentials. : *Biophys J.* 1989 Dec; 56(6):1053-69. Erratum in: *Biophys J* 1990 Mar; 57(3): following 684.

Russell C. Scaduto, Jr. and Lee W. Grotyohann. Measurement of mitochondrial membrane potential using fluorescent rhodamine derivatives. *Biophys J.* 1999 Jan; 76(1 Pt 1):469-77.

Rajagopal A, Pant AC, Simon SM, Chen Y. In vivo analysis of human multidrug resistance protein 1 (MRP1) activity using transient expression of fluorescently tagged MRP1. *Cancer Res.* 2002 Jan 15; 62(2):391-6.



# Section 4

## Section 4: Enzymatic Assays

# Fluorescent Monoamine Oxidase Detection Assay Kit – Fluoro MAO™

## Key Benefits

The Key Benefits of our the Fluoro MAO™ Kit are as follows:

- Non Radioactive
- Can monitor multiple time points to follow kinetics
- One-step, no wash assay
- Adaptable for High Throughput format
- Sensitive

## Assay Principle

Monoamine oxidase (MAO) is a flavin-containing enzyme that catalyses the oxidation of a variety of amine-containing neurotransmitters such as serotonin, norepinephrine, epinephrine and dopamine to yield the corresponding aldehydes (1). MAO exists in two isoforms, namely MAO-A and MAO-B, which are the products of two distinct genes (2).

MAO-A and B exhibit different specificities to substrates and inhibitor selectivities. Extensive studies have been performed to characterize their properties (3-7). MAO-A acts preferentially on serotonin and norepinephrine, and is inhibited by clorgyline. MAO-B acts preferentially on 2-phenylethylamine and benzylamine and is inhibited by deprenyl and pargyline.

Localized in the outer mitochondrial membrane, these enzymes are found throughout the body. Often only one form of the enzyme is present in a specific organ and/or within a specific cell type (8-9). In addition to their role in regulating neurotransmitters, these enzymes are also involved in processing biogenic amines (10) including tyramine (11).

The Fluoro MAO-A/B detection kit utilizes a non - fluorescent detection reagent to measure  $H_2O_2$  released from the conversion of a substrate to its aldehyde via MAO-A/B. Furthermore,  $H_2O_2$  oxidizes the detection reagent in a 1: stoichiometry to produce a fluorescent product resorufin. This oxidation is catalyzed by Peroxidase.

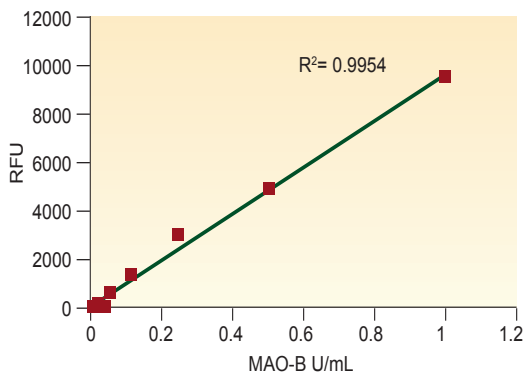
## Reactivity

Substrate +  $O_2$  +  $H_2O$  + MAO-A/B  $\longrightarrow$  aldehyde +  $NH_3$  +  $H_2O_2$

$H_2O_2$  + Detection reagent (non-fluorescent) + Peroxidase  $\longrightarrow$  Resorufin (fluorescent)

Excitation: 530-570 nm; Emission: 590-600 nm

The fluorescent monoamine oxidase detection kit can be used to monitor MAO activity and screen of MAO inhibitors



*In this figure, recombinant MAO-B was serially diluted in 1X Reaction Buffer. 100  $\mu$ L of diluted MAO-B per well was mixed with 100  $\mu$ L of Reaction Cocktail. The reaction was incubated at Room Temperature in the dark for 1 hour. Fluorescence was measured with a microplate reader using excitation at 530nm and fluorescence emission at 590nm.*

## Kit Contents

The Fluoro MAO™ Kit includes the following for 500 assays:

- Part #3011: 1 Bottel 5X Reaction Buffer pH 7.4
- Part #4007: 1 vial Detection Reagent
- Part #6005: 1 vial Horseradish Peroxide
- Part #7006: 1 vial MAO-B substrate Benzylamine
- Part #7005: 1 vial MAO-A/B Substrate: Tyramine
- Part #7003: 1 vial Pargyline Monoamine Oxidase D Inhibitor (12-14)
- Part #7002: 1 vial Clorgyline Monoamine Oxidase A Inhibitor (12-14)

## Ordering Information

The following Fluoro MAO™ Kit is available:

Catalog No..	Quantity	Price (in US dollars)
FLMAO100-3	500 Tests	\$245

## References

Waldmeier PC (1987) Amine oxidases and their endogenous substrates. *J Neural Transm Suppl* 23:55–72.

Bach, A. W. J., N. C. Lan, D. L. Johnson, C. W. Abell, M. E. Bembenc, S. W. Kwan, P. H. Seeburg & J. C. Shih: cDNA cloning of human liver monoamine oxidase A and B: molecular basis of differences in enzymatic properties. *Proc. Nat. Acad. Sci. U.S.A.* 1988, 85, 4934–4938.

Johnston, J. P.: Some observations upon a new inhibitor of mono-amine oxidase in brain tissue. *Biochem. Pharmacol.* 1968, 17, 1285–1297.

Suzuki, O., E. Noguchi & K. Yagi: A simple fluorometric assay for type B monoamine oxidase activity in rat tissues. *J. Biochem.* 1976, 79, 1297–1299.

Fowler, C. J. & B. A. Callingham: Substrate-selective activation of rat liver mitochondrial monoamine oxidase by oxygen. *Biochem. Pharmacol.* 1978, 27, 1995–2000.

Tipton, K. F.: Enzymology of monoamine oxidase. *Cell Biochem. Funct.* 1986, 4, 79–87.

Youdim, M. B. H. & M. Tenne: Assay and purification of liver monoamine oxidase. *Methods Enzymol.* 1987, 142, 617–626.

Trendelenburg U, Cassis L, Grohmann M and Langeloh A (1987) The functional coupling of neuronal and extraneuronal transport with intracellular monoamine oxidase. *J Neural Transm Suppl* 23:91–101.

Yu PH, Davis BA and Boulton AA (1992) Neuronal and astroglial monoamine oxidase: Pharmacological implications of specific MAO-B inhibitors. *Prog Brain Res* 94:309–315.

Strolin Benedetti M and Tipton KF (1998) Monoamine oxidases and related amine oxidases as phase I enzymes in the metabolism of xenobiotics. *J Neural Transm Suppl* 52:149–171.

Hauptmann N, Grimsby J, Shih JC and Cadenas E (1996) The metabolism of tyramine by monoamine oxidase A/B causes oxidative damage to mitochondrial DNA. *Arch Biochem Biophys* 335:295–304.

Methods Enzymology: Metabolism of Aromatic Amino Acids and Amines. Volume 142, page 617 (1997).13. Holt A.; Sharman D.F.; Baker G.B.; Palcic M.M. A Continuous Spectrophotometric Assay for Monoamine Oxidase and Related Enzymes in Tissue Homogenates *Analytical Biochemistry*, January 1997, vol. 244, no. 2, pp. 384-392(9).

D. W. R. Hall, Bridget W. Logan and G. H. Parsons. Further studies on the inhibition of monoamine oxidase by M & B 9302 (clorgyline)—I. Substrate specificity in various mammalian species. *Biochemical Pharma.*

# Fluorescent Semicarbazide-Sensitive Amine Oxidase Detection Kit - Fluoro SSAO™

## Key Benefits

The Key Benefits of our Fluoro SSAO™ Kit are as follows:

- Non Radioactive
- Can monitor multiple time points to follow kinetics
- Monitors Enzymatic Activity
- One-step, no wash assay
- Adaptable for High Throughput format
- Enzyme Positive Control included in kit

## Assay Principle

Semicarbazide-sensitive amine oxidase (SSAO) is a common name for a widely distributed enzyme in nature. In man this enzyme is present in the vascular system and circulates in plasma. SSAO differ from the monoamine oxidases A and B in substrate and inhibitor patterns. These enzymes have been widely studied and their tissue distribution, molecular properties, substrate specificities and inhibitor sensitivities are extensively reviewed (2, 3).

SSAO exists in two forms: tissue bound and soluble (plasma SSAO). Tissue bound SSAO activity is associated with blood vessels, mainly in smooth muscle layers, however it is also associated with spleen, placenta, bone marrow, kidney, sclera, retina, endothelial cells, adipocytes, chondrocytes and fibroblasts. (4, 5). Evidence suggests that plasma SSAO originates from the cleavage of membrane-bound form. The possible sources of plasma SSAO are still unclear, but it has been suggested that it may be derived from liver, retina, placenta and bone tissue (6, 7, 8).

SSAO's functional role has been suggested to be involved in: apoptosis, atherogenesis, cell adhesion, leucocyte trafficking, glucose transport and local production of hydrogen peroxide. Reports of elevated levels of SSAO have been reported in congestive heart failure, diabetes mellitus, Alzheimer's disease and various other inflammatory diseases (1).

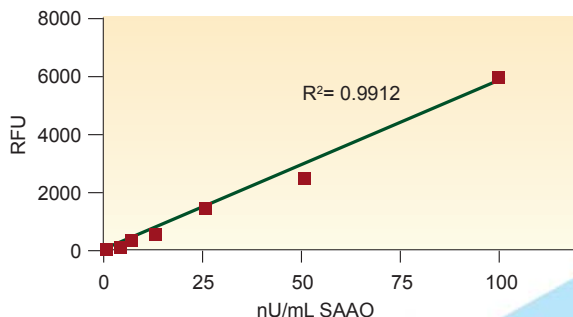
Furthermore byproducts of SSAO deamination, such as formaldehyde and methylglyoxal, have been proposed to be involved in pathogenesis of cancer, aging and atherosclerosis.

The Fluoro SSAO™ detection kit utilizes a non-fluorescent detection reagent, to measure H<sub>2</sub>O<sub>2</sub> released from the conversion of Benzylamine to Benzaldehyde via SSAO. Furthermore H<sub>2</sub>O<sub>2</sub> oxidizes the detection reagent in a 1:1 stoichiometry to produce a fluorescent product resorufin. This oxidation is catalyzed by Peroxidase.

## Reactivity



Excitation: 530–571 nm; Emission: 590–600 nm



*In this figure, bovine serum Semicarbazide-sensitive amine Oxidase was serially diluted in 1X Reaction buffer. The serially diluted samples were run as described in the protocol. The samples were read after a 3 hours incubation period.*

*Excitation: 530nm and emission: 590nm.*

## Kit Contents

The Fluoro SSAO™ Kit includes the following:

- Part #3011: 1 Bottel 5X Reaction Buffer pH 7.4
- Part #4007: 1 vial Detection Reagent
- Part #6005: 1 vial Horseradish Peroxide
- Part #7001: 1 vial SSAO substrate Benzylamine
- Part #6006: 1 vial SSAO Enzyme
- Part #7003: 1 vial Pargyline Monoamine Oxidase D Inhibitor (12-14)
- Part #7004: 1 vial Semicarbazide: Semicarbazide sensitive amine oxidase inhibitor

## Ordering Information

The following Fluoro SSAO™ Kit is available:

Catalog No..	Quantity	Price (in US dollars)
SSAO100-3	500 Tests	\$395

## References

Yayin Tahiri Kasim et. al., Turk J Biochem; 29 (3);247-254 (2004); Semicarbazide-Sensitive Amine Oxidase: Biochemical and Physiological Properties

B. A. Callingham, A.Holt, J. Elliott .J. Neural Transm. (Suppl.) 32, 279 –290 (1990).

B. A. Callingham and M. A. Barrand .J. Neural Transm. (Suppl.) 23, 37 –54 (1987).

G.A.Lyles.J.Neural Transm. (Suppl.) 41, 387 –396 (1994).

K. Magyar, Z. Mészáros and P. Mátyus. Semicarbazide-sensitive amine oxidase. Its physiological significance Pure Appl. Chem., Vol. 73, No.9, pp.1393 –1400,2001.

Boomsma F.,van Dijk J.,Bhaggoe U.M.,Bouhuizen A.M.B.,van den Meiracker A.H.(2000)Variation in semicarbazide-sensitive amine oxidase activity in plasma and tissues of mammals. Comp .Biochem.

Ekblom J.,Gronval J.L.,Garpenstrand H.,Nillson S.,Oreland L.(2000)Is semicarbazide-sensitive amine oxidase in blood plasma partly derived from the skeleton?. Neurobiology, 8,129-135.

McGuirl M.A., Dooley D.M. (1999) Copper-containing oxidases. Curr. Opin. Chem. Biol., 3,138-144.

Methods Enzymology 142, 617 (1997).

Holt A.; Sharman D.F.; Baker G.B.; Palcic M.M. A Continuous Spectrophotometric Assay for Monoamine Oxidase and Related Enzymes in Tissue Homogenates Analytical Biochemistry, January 1997, vol. 244, no. 2, pp. 384-392(9).

Biochem Pharmacol 18, 1447 (1969).

## Fluorescent Catalase Detection Assay Kit – Fluoro Catalase™

### Key Benefits

The Key Benefits of our the Fluoro Catalase™ Kit are as follows:

- Sensitive Fluorescent Assay
- Enzyme Positive Control included in kit
- Can monitor multiple time points to follow kinetics
- One-step, No Wash assay
- Adaptable for High Throughput format
- Monitors enzymatic activity
- Measurements on any standard Fluorescent Plate Reader

### Assay Principle

Catalase is an antioxidant enzyme that catalyses the decomposition of hydrogen peroxide ( $H_2O_2$ ) to water and oxygen. Catalase is ubiquitously expressed in mammalian and non-mammalian aerobic cells containing the cytochrome system. The enzyme has been isolated from various sources, including bacteria and plant cells (1-3). Catalase activity varies greatly from tissue to tissue. Highest activity is seen in liver and kidney, while lowest activity is seen in connective tissue (3). In eukaryotic cells, catalase is concentrated in organelles called peroxisomes (4).

The production of hydrogen peroxide in eukaryotic cells is an end product result of various oxidases and superoxide dismutase reactions. Accumulation of  $H_2O_2$  can result in cellular damage through oxidation of proteins, DNA and lipids thus resulting in cell death and mutagenesis (8-11).  $H_2O_2$  role in oxidative stress related diseases have been widely studied (8, 12).

The Fluoro Catalase™ detection kit is sensitive assay that utilizes a non - fluorescent detection reagent to measure  $H_2O_2$  substrate left over from the catalase reaction (5-6).

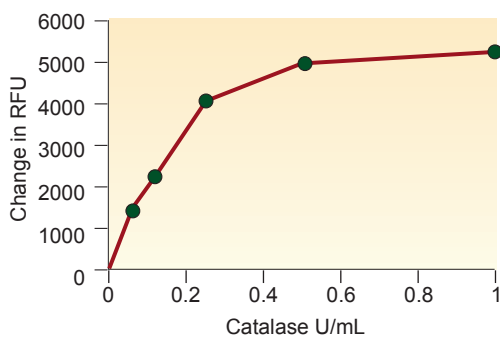
### Reactivity

$H_2O_2$  + Catalase  $\longrightarrow$  Water + oxygen



$H_2O_2$  (Left Over) + Detection reagent (non-fluorescent) + Peroxidase  $\longrightarrow$  Resorufin (fluorescent)

Excitation: 530-570 nm; Emission: 590-600 nm



*In this figure, catalase activity was detected using the Fluoro Catalase kit. The reaction contained 20uM H2O2 (final) per well and the indicated amounts of catalase in 1X reaction Buffer. The reaction was incubated for 30 minutes at room temperature. Next 100uL of Reaction cocktail was added to each well and the reaction incubated for another 10 minutes in the dark at room temperature. Fluorescence was measured at excitation 530nm and emission detected at 590nm. The graph reports the change in fluorescence, observed fluorescence from negative control (no catalase) minus catalase sample fluorescence.*

## Kit Contents

The Fluoro Catalase™ Kit includes the following for 500 assays:

- Part #3021: 1 Bottel 5X Reaction Buffer pH 7.4
- Part #4010: 5 vials Detection Reagent
- Part #6009: 1 vial Horseradish Peroxide
- Part #3022: 1 vial Hydrogen Peroxide
- Part #6008: 1 vial Catalase Enzyme

## Ordering Information

The following Fluoro Catalase™ Kit is available:

Catalog No..	Quantity	Price (in US dollars)
FLOCAT100-3	500 Tests	\$275

## References

Deisseroth, A., and Dounce, A.L. Catalase: Physical and chemical properties, mechanism of catalysis, and physiological role *Physiol. Rev.*, 50, 319-375 (1970).

Sebastian Mueller, Hans-Dieter Riedel and Wolfgang Stremmel. Determination of Catalase Activity at Physiological Hydrogen Peroxide Concentrations. *Analytical Biochemistry* Volume 245, Issue 1, 1 February 1997, Pages 55-60.

Deisseroth, A., and Dounce, A.L. Catalase: Physical and chemical properties, mechanism of catalysis, and physiological role. *Physiol. Rev.*, 50, 319-375 (1970).

Marcel Zámocký and Franz Koller. Understanding the structure and function of catalases: clues from molecular evolution and in vitro mutagenesis. *Progress in Biophys. Mol. Biol.*, 72, 19-66 (1999).

Mingjie Zhou, Zhenjun Diwu, Nataliya Panchuk-Voloshina and Richard P. Haugland. A Stable Nonfluorescent Derivative of Resorufin or the Fluorometric Determination of Trace Hydrogen Peroxide: Applications in Detecting the Activity of Phagocyte NADPH Oxidase and Other Oxidases. *Anal Biochem* 253, 162 (1997).

J. G. Mohanty, Jonathan S. Jaffe, Edward S. Schulman and Donald G. Raible. A highly sensitive fluorescent micro-assay of H<sub>2</sub>O<sub>2</sub> release from activated human leukocytes using a dihydroxyphenoxazine derivative. *J. Immunol Methods* 202, 133 (1997).

Tatyana V. Votyakova, Ian J. Reynolds. Detection of hydrogen peroxide with Amplex Red: interference by NADH and reduced glutathione auto-oxidation. *Archives of Biochemistry and Biophysics*, 431:138-144 (2004).

Jingxiang Bai, Ana M. Rodriguez, J. Andres Melendez, and Arthur I. Cederbaum. Overexpression of Catalase in Cytosolic or Mitochondrial Compartment Protects HepG2 Cells against Oxidative Injury. *J. Biol. Chem.*, Sep 1999; 274: 26217 - 26224

Tada-Oikawa, S. et al., *FEBS Lett*, 442, 65-69 (1999).

Hampton, M.B., and Orrenius, S., *FEBS Lett*, 414, 552-556 (1997).

Kowaltowski, A.J. et al., *FEBS Lett*, 473, 177-182 (2000).

Tome, M.E. et al., *Cancer Res.*, 61, 2766-2733 (2001).

## Myeloperoxidase Detection Kit – Fluoro MPO™

### Key Benefits

The Key Benefits of our the Fluoro MPO™ Kit are as follows:

- Non-Radioactive
- Can monitor multiple time points to follow kinetics.
- One-step, no wash assay.
- Adaptable for High Throughput format
- Highly Sensitive
- Fluorescence or Absorbance Readout

### Assay Principle

Myeloperoxidase (MPO) is a highly cationic glycosylated hemoprotein that has a molecular weight of 144kD. The hemoprotein consists of two dimers linked via a disulfide bridge. Each dimer is composed of a heavy (53kD) and light (15kD) subunit. Each heavy chain contains an independently acting protoporphyrin group containing a central iron (1-5). MPO is present in the azurophilic granules of polymorphonuclear leukocytes (PMNs) and is unique to neutrophils and monocytes. However, monocytes contain only one third of the MPO found in PMNs. MPO utilizes  $H_2O_2$  produced by the neutrophils to oxidize a variety of aromatic compounds to give substrate radicals for bactericidal activity (4 reviews). This enzyme is unique however in that it can oxidize chloride ions to produce a strong nonradical oxidant, HOCl. HOCl is the most powerful bactericidal produced by neutrophils (4 reviews). Excessive production of these radicals can cause oxidative stress leading to oxidative tissue injury.

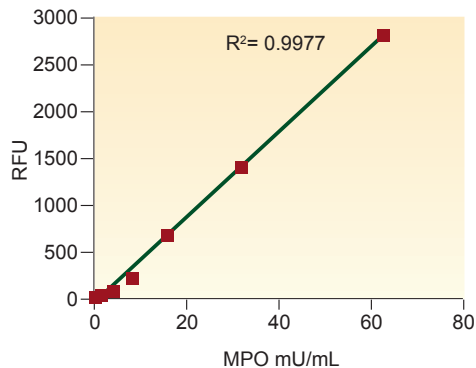
### Reactivity

$H_2O_2$  + Detection reagent (non-fluorescent) + MP  $\longrightarrow$  fluorescent analog.  
Excitation: 530-571 nm; Emission: 590-600 nm

### Applications

Fluoro MPO Kit™ applications include the following:

- Detection of MPO activity in neutrophils and macrophages
- Detection of PMN infiltration in tissue samples (inflammation and innate host defense mechanisms)
- Acute and chronic inflammatory disorders due to oxidative tissue damage
- MPO activity in acute and chronic manifestations of cardiovascular disease



*In this figure, the MPO standard curve was serially diluted in 1X Reaction Buffer. Reaction cocktail (RC) was prepared as described (without EPO inhibitor). Next 50?L of MPO standard and 50?L of RC was added to individual well of 96- well black plates. The plate was incubated at room and temperature in the dark. Data collected Ex:530nm, Em:590nm*

## Kit Contents

The Fluoro MPO™ Kit contents include the following:

- Part #3022: 10X Reaction Buffer: 60 ml pH 7.4
- Part #4007: Detection reagent: One vial for 500 assays.
- Part #3012: Hydrogen Peroxide: 1000µL of a stabilized 3% solution.
- Part #6015: MyeloPeroxidase: 1 vial at 30units/ml

## Ordering Information

The following Fluoro MPO™ Kit is available:

Catalog No..	Quantity	Price (in US dollars)
FLMPO100-3	500 Tests	\$425

## References

- Waldmeier PC (1987) Amine oxidases and their endogenous substrates. *J Neural Transm Suppl* 23:55–72.
- Bach, A. W. J., N. C. Lan, D. L. Johnson, C. W. Abell, M. E. Bembenck, S. W. Kwan, P. H. Seeburg & J. C. Shih: cDNA cloning of human liver monoamine oxidase A and B: molecular basis of differences in enzymatic properties. *Proc. Nat. Acad. Sci. U.S.A.* 1988, 85, 4934–4938.
- Johnston, J. P.: Some observations upon a new inhibitor of mono-amine oxidase in brain tissue. *Biochem. Pharmacol.* 1968, 17, 1285–1297.
- Suzuki, O., E. Noguchi & K. Yagi: A simple fluorometric assay for type B monoamine oxidase activity in rat tissues. *J. Biochem.* 1976, 79, 1297–1299.
- Fowler, C. J. & B. A. Callingham: Substrate-selective activation of rat liver mitochondrial monoamine oxidase by oxygen. *Biochem. Pharmacol.* 1978, 27, 1995–2000.
- Tipton, K. F.: Enzymology of monoamine oxidase. *Cell Biochem. Funct.* 1986, 4, 79–87.
- Youdim, M. B. H. & M. Tenne: Assay and purification of liver monoamine oxidase. *Methods Enzymol.* 1987, 142, 617–626.
- Trendelenburg U, Cassis L, Grohmann M and Langeloh A (1987) The functional coupling of neuronal and extraneuronal transport with intracellular monoamine oxidase. *J Neural Transm Suppl* 23:91–101.
- Yu PH, Davis BA and Boulton AA (1992) Neuronal and astroglial monoamine oxidase: Pharmacological implications of specific MAO-B inhibitors. *Prog Brain Res* 94:309–315.
- Strolin Benedetti M and Tipton KF (1998) Monoamine oxidases and related amine oxidases as phase I enzymes in the metabolism of xenobiotics. *J Neural Transm Suppl* 52:149–171.
- Hauptmann N, Grimsby J, Shih JC and Cadenas E (1996) The metabolism of tyramine by monoamine oxidase A/B causes oxidative damage to mitochondrial DNA. *Arch Biochem Biophys* 335:295–304.
- Methods Enzymology: Metabolism of Aromatic Amino Acids and Amines. Volume 142, page 617 (1997).
- Holt A.; Sharman D.F.; Baker G.B.; Palcic M.M. A Continuous Spectrophotometric Assay for Monoamine Oxidase and Related Enzymes in Tissue Homogenates *Analytical Biochemistry*, January 1997, vol. 244, no. 2, pp. 384-392(9).
- D. W. R. Hall, Bridget W. Logan and G. H. Parsons. Further studies on the inhibition of monoamine oxidase by M & B 9302 (clorgyline)—I. Substrate specificity in various mammalian species. *Biochemical Pharma*

# Acetylcholinesterase Detection in RBC, Saliva, lysates – Fluoro AChE™

## Key Benefits

The Key Benefits of our the Fluoro AChE™ Kit are as follows:

- Non Radioactive
- Can monitor multiple time points to follow kinetics.
- One-step, no wash assay.
- Adaptable for High Throughput format.
- Versatile: can detect acetylcholinesterase activity in RBC's, saliva & tissue lysates.

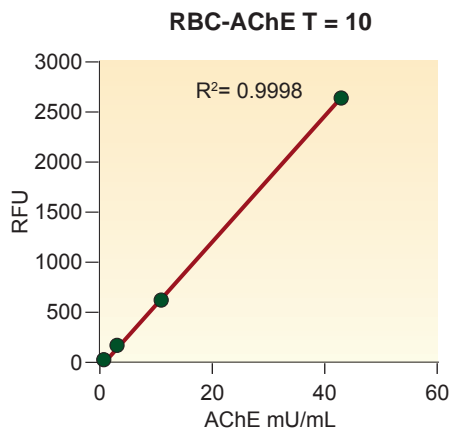
## Assay Principle

We have developed a highly sensitive, very rapid, extremely simple assay to determine acetylcholinesterase activity in RBC's, using the natural substrate, acetylcholine. Additionally, by using specific inhibitors, the kit can be used to detect AChE activity in a variety of samples. A series of coupled enzyme reactions quickly translates the presence of active AChE into a change in the fluorescence of a quenched detection reagent.

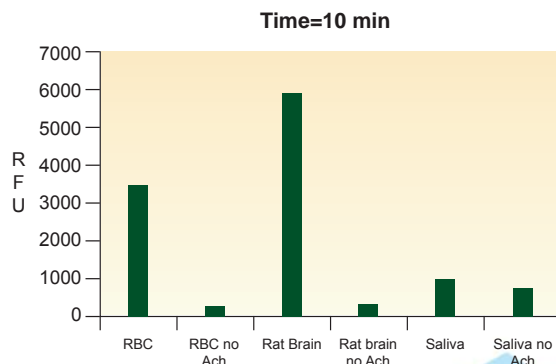
## Reactivity

AChE + ATP + H<sub>2</sub>O + coupled enzyme reaction + quenched dye  $\longrightarrow$  Fluorescent

Excitation: 530-570 nm; Emission: 590-600 nm



*Fig.1 Red Blood Cell AChE (RBC-AChE) was purified and protein concentration determined using the BCA Protein Assay Kit (Pierce). The RBC-AChE was titrated in 1X reaction buffer and activity determined using the Fluoro: AChE kit. Acetylcholine concentration = 1mM final. In the graph the background value has been subtracted (0 RBC-AChE) to generate standard curve.*



*Fig.2 Protine concentration for eash sample was determined using the BCA Protine Assay Kit (Pierce). The lysates were diluted in 1X reaction buffer and the indicated volumes add to the Fluoro AChE reaction (component A+B). Samples were incubated at room temperature in the dark for 10 minutes. Fluorescence was measured in a 96 well plate reader Ex: 530 nm Em: 590 nm. (ND = Not Determined). Acetylcholine: 1mM final.*

\*Control 2 (background) subtracted from signal.

## Kit Contents

The Fluoro AChE™ Kit contents include the following:

- Part #3042: Component A: Detection Reagent Diluent, 5.5mL
- Part #3043: Component B: Coupled enzyme reagent, 5.5mL
- Part #4016: Component C: 1vial detection reagent
- Part #3011: Component D: 5X Reaction Buffer
- Part #7012: Component E: Acetylcholine
- Part #6020: Component E: Red Blood Cell Acetylcholinesterase

## Ordering Information

The following Fluoro AChE™ Kit is available:

Catalog No.	Quantity	Price (in US dollars)
ACHE100-2	100 Tests	\$395
ACHE100-3	500 Tests	\$1,595

## References

Politoff, A., Blitz, A., and Rose, S.: Incorporation of Acetylcholinesterase Into Synaptic Vesicles is Associated with Blockade of Synaptic Transmission, *Nature* 256, 324, 1975

Friedenberg, R., and Seligman, A.: Acetylcholinesterase at the Myoneural Junction: Cytochemical Ultrastructure and Some Biochemical Considerations, *J Histochem Cytochem* 20, 771, 1972

Nachmansohn, D.: Proteins in Excitable Membranes, *Science* 168, 1059, 1970.

HA Berman and MM Decker. Kinetic, equilibrium, and spectroscopic studies on dealkylation ("aging") of alkyl organophosphonyl acetylcholinesterase. Electrostatic control of enzyme topography. *J. Biol. Chem.*, Aug 1986; 261: 10646-10652 .

Arie Ordentlich et al. The Architecture of Human Acetylcholinesterase Active Center Probed by Interactions with Selected Organophosphate Inhibitors. *J. Biol. Chem.*, May 1996; 271: 11953-11962.

Levy R. Tetrahydroaminoacridine and Alzheimer's disease. *Lancet*, 1987 Feb 7;1(8528):322.

Bolognesi ML et al. Propidium-based polyamine ligands as potent inhibitors of acetylcholinesterase and acetylcholinesterase-induced amyloid-beta aggregation. *J Med Chem*. 2005 Jan 13;48(1):24-7.

Schallreuter KU et al. Activation/deactivation of acetylcholinesterase by H2O2: more evidence for oxidative stress in vitiligo. *Biochem Biophys Res Commun*. 2004 Mar 5;315(2):502-8.

Nigg HN, Knaak JB. Blood cholinesterases as human biomarkers of organophosphorus pesticide exposure. *Rev. Environ. Contam. Toxicol*, 2000;163: p29-111.

## Chlorination and Peroxidation Detection – Fluoro MPOHOCL™

### Key Benefits

The Key Benefits of our the Fluoro MPOHOCL™ Kit are as follows:

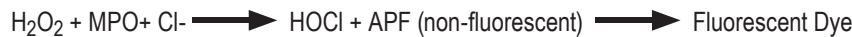
- Readout: Fluorescence
- Can monitor multiple time points to follow kinetics
- One-step, no wash assay.
- Adaptable for High Throughput format
- Sensitive

### Assay Principle

Myeloperoxidase (MPO) is a highly cationic glycosylated hemoprotein that has a molecular weight of 144kD. The hemoprotein consists of two dimers linked via a disulfide bridge. Each dimer is composed of a heavy (53kD) and light (15kD) subunit. Each heavy chain contains an independently acting protoporphyrin group containing a central iron (1-5). MPO is present in the azurophilic granules of polymorphonuclear leukocytes (PMNs) and is unique to neutrophils and monocytes. However, monocytes contain only one third of the MPO found in Pan's. MPO utilizes H<sub>2</sub>O<sub>2</sub> produced by the Europhiles to oxidize a variety of aromatic compounds to give substrate radicals for bactericidal activity (4 review). This enzyme is unique however in that it can oxidize chloride ions to produce a strong nonradical oxidant, HOCl. HOCl is the most powerful bactericidal produced by neutrophils (4 review). Excessive production of these radicals can cause oxidative stress leading to oxidative tissue injury (6).

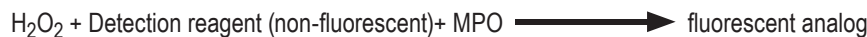
### Reactivity

#### Chlorination Reaction:



Excitation: 488 nm; Emission: 515-530 nm

#### Peroxidation Reaction:



Excitation: 530-571 nm; Emission: 590-600 nm

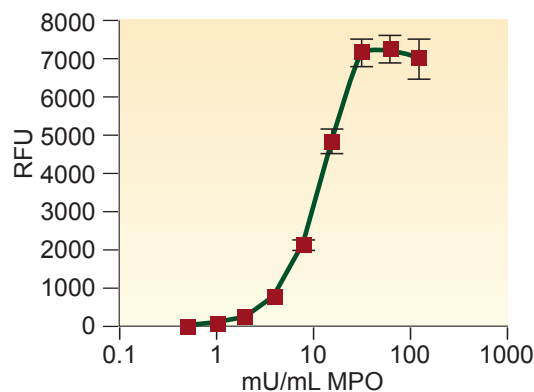
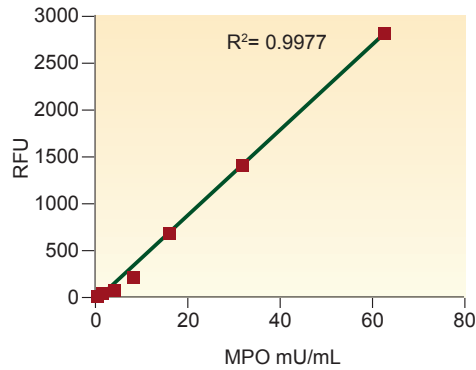


Fig.2 Red Blood Cell AChE (RBC-AChE) was purified and protein concentration determined using the BCA Protein Assay Kit (Pierce). The RBC-AChE was titrated in 1X reaction buffer and activity determined using the Fluoro: AChE kit. Acetylcholine concentration = 1mM final. In the graph the background value has been subtracted (0 RBC-AChE) to generate standard curve.



*In this figure, the MPO standard curve was serially diluted in 1X Reaction Buffer. Reaction cocktail (RC) was prepared as described (without EPO inhibitor). Next 50 $\mu$ L of MPO standard and 50 $\mu$ L of RC was added to individual well of 96-well black plates. The plate was incubated at room and temperature in the dark. Data collected Ex:530nm, Em:590nm*

## Kit Contents

The Fluoro MPOHOCL™ Kit contents include the following:

- Part #3002: 10X Assay Buffer: 60ml pH 4.7
- Part #4007: Detection Reagent: One vial, for 500 assays
- Part #3012: Hydrogen Peroxide: 1000 $\mu$ L of a stabilized 3% solution
- Part #6015: MyeloPeroxidase - 1 vial at 30units/ml
- Part #4011: APF

## Ordering Information

The following Fluoro MPOHOCL™ Kit is available:

Catalog No..	Quantity	Price (in US dollars)
MPOHOCL100-3	Perioxidation: 500 Reactions Chlorination: 290 Reactions	\$645

## References

Olsen, R. L. & Little, c. (1983) Biochem. J. 209, 781-787.

Nauseef, W. M., and Malech, H. L. (1986) Blood, 67, 1504-1507.

Andrews, P. C., Parnes, C., and Krinsky, N. I. (1984) Arch. Biochem. Biophys., 228, 439-442.

Mark B. Hampton, Anthony J. Kettle, and Christine C. Winterbourn. Inside the Neutrophil Phagosome: Oxidants, Myeloperoxidase, and Bacterial Killing. Blood, Vol. 92 No. 9 (November 1), 1998: pp. 3007-3017.

Andrews, P.C., Parnes, C. & Krinsky, N.I. (1984) Comparison of myeloperoxidase and hemi-myeloperoxidase with respect to catalysis, regulation, and bacterial activity. Arch. Biochem. Biophys. 228, 439-442.

Ken-ichi Setsukinai, Yasuteru Urano, Katsuko Kakinuma, Hideyuki J. Majima, and Tetsuo Nagano. Development of Novel Fluorescence Probes That Can Reliably Detect Reactive Oxygen Species and Distinguish Specific Species. THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 278, No. 5, Issue of January 31, pp. 3170-3175, 2003

# Eosinophil Peroxidase Detection Kit - Fluoro EPOTM

## Key Benefits

The Key Benefits of our Fluoro EPOTM are as follows:

- Readout: Fluorescence or absorbance.
- Can monitor multiple time points to follow kinetics.
- One-step, no wash assay.
- Adaptable for High Throughput format.

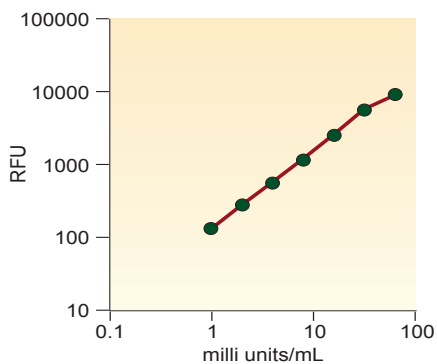
## Assay Principle

Eosinophil peroxidase (EPO) is the most abundant enzyme found in eosinophils. It is the major cytotoxic agent released by activated eosinophils and uses hydrogen peroxide to generate reactive oxidants from halides and pseudo halide thiocyanate (1,2). Eosinophils peroxidase has been shown to have antimycobacterial activity (3), however it is also implicated in tissue damage that occurs in asthma and other diseases (4,5). Currently, the function of eosinophil involvement in the immune response is being redefined. Once considered a cell involved in host protection of parasitic infection, eosinophils multiple functions as leukocytes involved in the initiation and propagation of diverse inflammatory responses are being investigated. Eosinophils are further involved as modulators of innate and adaptive immunity (6).

## Reactivity

$H_2O_2$  + Detection reagent (non-fluorescent) + EPO  $\longrightarrow$  fluorescent analog.

Excitation 530-571 nm; Emission 590 – 600 nm



*Figure 1. Eosinophil peroxidase was titrated in 1X reaction buffer and 50uL of each point was added in triplicate to 96 black wells. 50uL of reaction cocktail was added and the reaction was incubated in the dark at room temperature for 30 minutes.*

*Fluorescence was read at 530 nm excitation and emission detected at 590 nm.*

## Kit Contents

The Fluoro EPOTM Kit contents include the following:

- Part #3002: 10X Assay Buffer: 60ml pH 4.7
- Part #4016: Detection Reagent: One vial for 100 assays
- Part #3012: Hydrogen Peroxide: 1000μL of a stabilized 3% solution
- Part #6016: Eosinophil Peroxidase: 100μL in 1 vial at 10 units/ml

## Ordering Information

The following Fluoro EPOT™ Kit is available:

Catalog No..	Quantity	Price (in US dollars)
FLEPO100-2	100 Tests	\$445

## References:

- Mayeno, A. N., Curran, A. J., Roberts, R. L. and Foote, C. S. (1989) Eosinophils preferentially use bromide to generate halogenating agents. *J. Biol. Chem.* 264, 5660±5668
- Slungaard, A. and Mahoney, J. R. (1991) Thiocyanate is the major substrate foreosinophil peroxidase in physiologic fluids. *J. Biol. Chem.* 266, 4903±4910 (Pruitt, K. M. and Tenovuo, J. V., eds.), pp. 31±53, Marcel Dekker, New York
- Human Eosinophil Peroxidase Induces Surface Alteration, killing, and lysis of *Mycobacterium tuberculosis*", *Infection and Immunity*, Feb. 2003, p. 605-613 by The American Society for Microbiology.
- Bousquet, J., Chanez, P., Lacoste, J. Y., Barneon, G., Ghavanian, N., Enander, I., Venge, P., Ahlstedt, S., Simony-Lafontaine, J., Godard, P. and Michel, F. (1990) Eosinophilic inflammation in asthma. *New Engl. J. Med.* 323, 1033±1039
- Parrillo, J. E., Borer, J. S., Henry, W. L., Wolff, S. M. and Fauci, A. S. (1979) The cardiovascular manifestations of the hypereosinophilic syndrome. Prospective study of 26 patients, with review of the literature. *Am. J. Med.* 67, 572-582
- Marc E. Rothenberg and Simon P. Hogan.. THE EOSINOPHIL Annual Review of Immunology Vol. 24: 147-174 (Volume publication date April 2006.)
- Mingjie Zhou, Zhenjun Diwu, Nataliya Panchuk-Voloshina and Richard P. Haugland. A Stable Nonfluorescent Derivative of Resorufin for the Fluorometric Determination of Trace Hydrogen Peroxide: Applications in Detecting the Activity of Phagocyte NADPH Oxidase and Other Oxidases. *Anal Biochem* 253, 162 (1997).
- J. G. Mohanty, Jonathan S. Jaffe, Edward S. Schulman and Donald G. Raible. A highly sensitive fluorescent micro-assay of H<sub>2</sub>O<sub>2</sub> release from activated human leukocytes using a dihydroxyphenoxazine derivative. *J. Immunol Methods* 202, 133 (1997).
- Tatyana V. Votyakova and Ian J. Reynolds. Membrane Potential dependent and -independent production of reactive oxygen species by rat brain mitochondria. *J Neurochem* 79, 266 (2001).
- Chun Song, Abu B. Al-Mehdi, and Aron B. Fisher. An immediate endothelial cell signaling response to lung ischemia. *Am J Physiol Lung Cell Mol Physiol* 281, L993 (2001).
- Samantha C. Richer and W.C.L. Ford. A critical investigation of NADPH oxidase activity in human spermatozoa. *Mol Hum Reprod* 7, 237 (2001).
- William G. Gutheil, Miglena E. Stefanova and Robert A. Nicholas. Fluorescent Coupled Enzyme Assays for -Alanine: Application to Penicillin-Binding Protein and Vancomycin Activity Assays. *Anal Biochem* 287, 196 (2000).
- Dominik Peus, Remus A. Vasa, Astrid Beyerle, Alexander Meves, Carsten Krautmacher and Mark R. Pittelkow. UVB Activates ERK1/2 and p38 Signaling Pathways via Reactive Oxygen Species in Cultured Keratinocytes. *J Invest Dermatol* 112, 751 (1999).
- Tatyana V.Votyakova ,Ian J.Reynolds. Detection of hydrogen peroxide with Amplex Red:interference by NADH and reduced glutathione auto-oxidation. *Archives of Biochemistry and Biophysics*, 431: 138-144 (2004).
- D. W. R. Hall, Bridget W. Logan and G. H. Parsons. Further studies on the inhibition of monoamine oxidase by M & B 9302 (clorgyline)—I . Substrate specificity in various mammalian species. *Biochemical Pharma*

# Section 5

## Section 5: Metabolic Assays

## Fluorescent Thiol Detection Kit - Fluoro Thiol™

### Key Benefits

The Key Benefits of our Fluoro Thiol™ Detection Kit are:

- Detection of Reduced thiols in cells or tissue extracts.
- Sensitive fluorescent assay.
- Detection of reduced thiol levels in apoptosis, metabolism and oxidative stress.

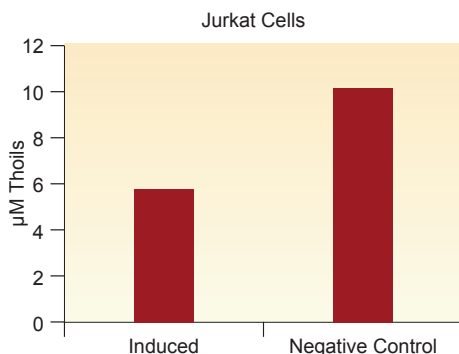
### Assay Principle

During the normal course of metabolism, oxygen is partly reduced as electrons leak out of the electron transport chain during respiration. These partially reduced oxygen species (ROS) can react with organic substances through non-catalytic means. Furthermore, ROS can be generated via endogenous enzyme systems like plasma NADPH oxidase, cytoplasmic xanthine oxidase and organelle sources e.g., cytochrome P-450. ROS have been implicated in regulating diverse cellular functions including proliferation, defense against pathogens, intra-cellular signaling, transcriptional activation and apoptosis. Elevation of ROS beyond the buffering capacity of the cell can lead to oxidative stress. Elevated ROS levels can lead to damage of DNA/RNA, proteins and lipids which may lead to apoptosis. Cells have developed several mechanisms to counter act elevated ROS levels such as a thiol reducing buffer composed of cellular thiol levels (glutathione and thioredoxin) for the maintenance of the reduction-oxidation (redox) state of the cell, and enzymes to remove ROS (catalase, superoxide dismutase and glutathione peroxidase) (1-2).

### Reaction

Cell Technology's Fluoro Thiol kit detects Thiol levels in cells and tissue extracts. Hatsuo Maeda and co-workers have developed a quenched dye as a fluorescent specific probe for general thiol detection (3). The reaction scheme is outlined below.

Thiol (reduced) + non-fluorescent Dye  $\longrightarrow$  fluorescent analog excitation at 488 nm and emission at 515-530 nm



*Jurkat cells were incubated with 1 mM staurosporine (4) for 3 hours. After which thiol levels were quantified using Cell Technology's Fluoro Thiol kit. The graph represents approximately  $1 \times 10^4$  cells per reaction ( $n=3$ ).*

### Kit Contents

The Fluoro Thiol™ Kit contents include the following:

- Part #4021: DYE
- Part #3053: Lysis Buffer
- Part #7015: GSH Standard, 3 vials

## Ordering Information

The following Fluoro Thiol™ kits are available:

Version/Catalog No.	Quantity	Price (in US dollars)
FLTHIO100-2	100 Tests	\$445

## References:

Gamaley IA and Klyubin IV (1999) Roles of reactive oxygen species: Signaling and regulation of cellular functions. *Int Rev Cytol* 188:203–238..

Nakamura H, Nakamura K and Yodoi J (1997) Redox regulation of cellular activation. *Annu Rev Immunol* 15:351–369.

2,4-Dinitrobenzenesulfonyl Fluoresceins as Fluorescent Alternatives to Ellman's Reagent in Thiol-Quantification Enzyme Assays\*. Hatsu Maeda,\*Hiromi Matsuno, Mai Ushida, Kohei Katayama, Kanako Saeki, and Norio Itoh. *Angew.Chem.Int.Ed.* 2005, 44, 2922 – 2925

Marchetti, P., et al., Redox regulation of apoptosis: impact of thiol oxidation status on mitochondrial function. *Eur.J.Immunol.*, 27, 289- 296 (1997).

## Fluorescent NAD/NADH Detection Kit - Fluoro NAD™

### Key Benefits

The Key Benefits of our Fluoro NAD™ are as follows:

- Detection of NAD/NADH activity in cells or tissue extracts.
- Detection of NAD/NADH levels in apoptosis, metabolism, proliferation, DNA repair, senescence, endocrine signaling and life span.
- NAD/NADH detection in Bacterial, fungal and plant cells.
- Readout: Fluorescence

### Assay Principle

The role of pyridine nucleotides (NAD/NADH) metabolism in health is of continual and increased interest. A growing amount of evidence supports the fact that NAD metabolism regulates important biological effect including life span. NAD, through poly-ADP-ribosyl polymerase (PARP), mono-ADP-ribosyltransferase (ARTs) and recently characterized sirtuin enzymes, exerts potential biological effects. These enzymes modify proteins to regulate their function via ADPribosylation or deacetylation and are involved in several pathways including apoptosis, DNA repair, senescence and endocrine signaling. This suggests that either the enzymes or NAD could be an important therapeutical target (1).

The Fluoro NAD/NADH™ detection kit utilizes a non-fluorescent detection reagent, which is oxidized in the presence NADH to produce its fluorescent analog and NAD. NAD is further converted to NADH via an enzyme coupled reaction. The enzyme reaction specifically reacts with NAD/NADH and not with NADP/NADPH.

### Reactivity

1. NADH + non-fluorescent detection reagent+ electron coupler  $\longrightarrow$  fluorescent analog + NAD

2. NAD + enzyme coupled reaction  $\longrightarrow$  NADH (then proceeds to reaction 1).

Excitation: 530-570 nm; Emission at 590-600 nm

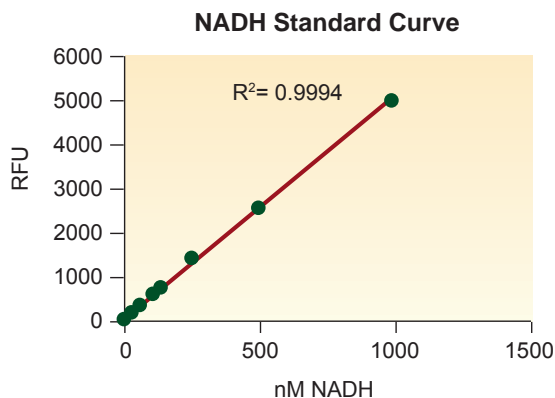


Figure 1: NADH standard curve titrated in NAD/NADH lysis buffer. Incubation time = 1 hour.

### Kit Contents

The Fluoro NAD™ Kit contents include the following:

- Part #4018: NADH Detection Reagent
- Part #7013: NADH Standard
- Part #3044: NAD/NADH Reaction Buffer
- Part #3045: NAD/NADH Lysis Buffer
- Part #3046: NAD Extraction Buffer
- Part #3047: NADH Extraction Buffer
- Part #3051: NADH Standard Diluent

## Ordering Information

The following Fluoro NAD™ kits are available:

Catalog No.	Quantity	Price (in US dollars)
FLNADH100-2	100 Tests	\$445
FLNADH100-3	500 Tests	\$2,095

## References:

Anthony A. Sauve NAD+ and Vitamin B3: From metabolism to therapies - J. Pharmacol. Exp. Ther. 2007 : jpet.107.120758v1

# Fluorescent NADP/NADPH Detection Kit - Fluoro NADPH™

## Key Benefits

The Key Benefits of our Fluoro NADPH™ are as follows:

- Detection of NADP/NADH content in cells or tissue extracts.
- Study of NADP/NADPH levels antioxidation and oxidative stress
- Detection NADP/NADPH in cell death, energy metabolism, mitochondria
- Species Independent - NADP/NADPH detection in Bacterial, fungal and plant cells
- Highly Sensitive – Detects up to 4 nM NADP and NADPH
- Highly Specific - No Cross reactivity with NAD/NADH
- Readout – 96 well Fluorescent Plate reader readout

## Assay Principle

Nicotinamide adenine dinucleotide phosphate (NADP<sup>+</sup>) is used in anabolic reactions, such as lipid and nucleic acid synthesis, which require NADPH as a reducing agent. NADPH is the reduced form of NADP<sup>+</sup>, and NADP<sup>+</sup> is the oxidized form of NADPH. In cells, NADPH plays the role of a carrier of reducing power and is primarily involved in maintaining optimal redox metabolism. A simplified assay for the measurement of NAD and NADP is critical to understanding the roles of these pyridine nucleotides in normal and abnormal cells.

NADPH is produced in the oxidative phase of the pentose phosphate pathway in cells, a multifunctional pathway whose primary purpose is to generate reducing power in the form of NADPH. NADPH is a cofactor for enzymes that synthesize energy-rich molecules and provide the reducing equivalents for the oxidation-reduction involved in protecting the cell from the toxicity of reactive oxygen species (ROS) and NADPH oxidase-dependent ROS generation. Both NAD and NADP have been shown to influence hemoglobin affinity for oxygen in erythrocytes. In plant cells, NADPH is used as the reducing power for the biosynthetic reactions in the Calvin cycle of photosynthesis (1-2).

Cell Technology's Fluoro NADPH™ provides a highly reliable, sensitive fluorometric assay for the quantification of NADP, NADPH and their ratio in biological samples.

## Reaction

The Fluoro NADPH™ detection kit utilizes a non-fluorescent detection reagent, which is oxidized in the presence NADPH to produce its fluorescent analog and NADP. NADP is further converted to NADPH via an enzyme-coupled reaction. The enzyme reaction specifically reacts with NADP/NADPH and not with NAD/NADH.

1. NADPH + non-fluorescent detection reagent + electron coupler → fluorescent analog + NADP
2. NADP + enzyme coupled reaction → NADPH (then proceeds to reaction 1).

Excitation: 530-570 nm and Emission at 590-600 nm

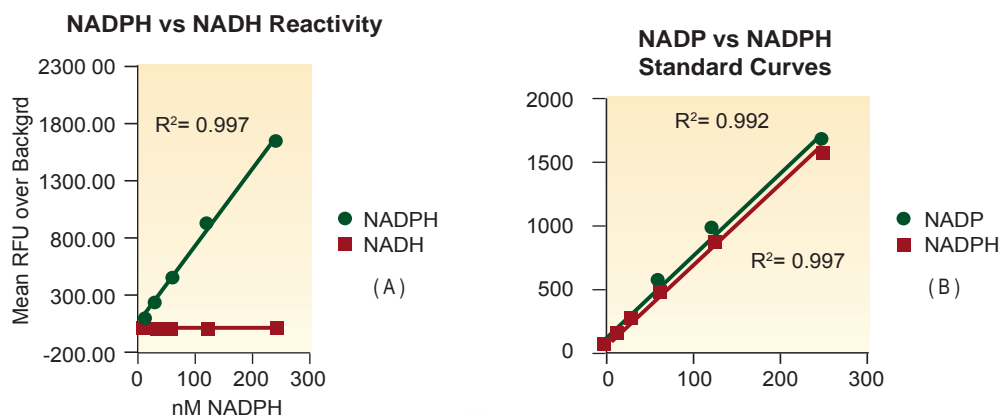


Figure 1. Comparison of NADPH vs NADH Standard Curves (graph A) and NADP vs NADPH Standard curves (graph B).

## Kit Contents

The Fluoro NADP™ Kit contents include the following:

- Part #6022: Enzyme Mix
- Part #3050: NADP/NADPH Lysis Solution
- Part #3046: NADP Extraction Buffer
- Part #3047: NADPH Extraction Buffer
- Part #3049: Reaction Buffer
- Part #3052: Standard Curve Diluent
- Part #4019: NADPH Detection Reagent
- Part #3048: 3X Substrate Mix
- Part #7014: NADPH Standard

## Ordering Information

The following Fluoro NADP™ kits are available:

Version/Catalog No.	Quantity	Price (in US dollars)
NADPH100-2	100 Tests	\$495
NADPH100-3	500 Tests	\$2,375

## References

Comprehensive Invited Review WEIHAI YING. NAD /NADH and NADP /NADPH in Cellular Functions and Cell Death: Regulation and Biological Consequences. ANTIOXIDANTS & REDOX SIGNALING Volume 10, Number 2, 2008.

Bedard K and Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev* 87:245–313, 2007.

Lowry, Oliver H., Passonneau, Janet V. and Rock, Martha K. The Stability of Pyridine Nucleotides. *The Journal of Biological Chemistry*, 236, #10, 1961.

# Total Cholesterol Detection Kit - Fluoro Cholesterol™

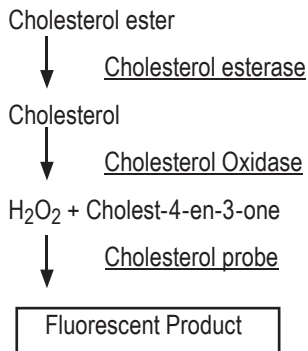
## Key Benefits

The Key Benefits of our Fluoro Cholesterol™ Detection kit are as follows:

- Detection of cholesterol in biological samples
- Study effects of drugs on cholesterol metabolism
- Highly Sensitive – Detects up to 200 nM cholesterol
- Measures total cholesterol in a simple, one-step reaction
- Readout – Absorbance, Fluorescence

## Assay Principle

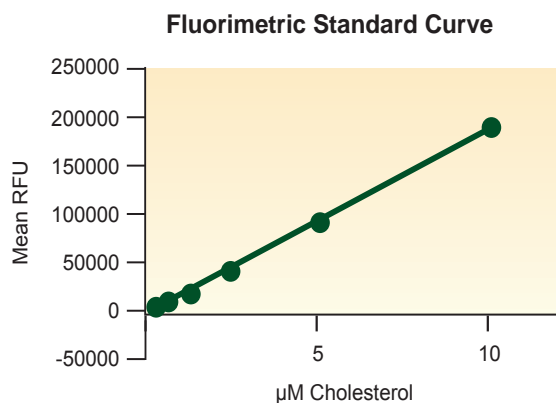
Cell Technology's Fluoro Cholesterol™ assay kit provides a simple, one-step fluorimetric or colorimetric method for determination of total cholesterol in serum and plasma samples. The assay is based on an enzyme-coupled reaction that detects both free cholesterol and cholesterol esters. Cholesterol esters are hydrolyzed by cholesterol esterase into cholesterol, which is then oxidized by cholesterol oxidase to yield hydrogen peroxide and cholest-4-en-3-one (ketone). The hydrogen peroxide then reacts with the cholesterol probe (detection reagent) in a 1:1 stoichiometry to produce the stable fluorescent product.



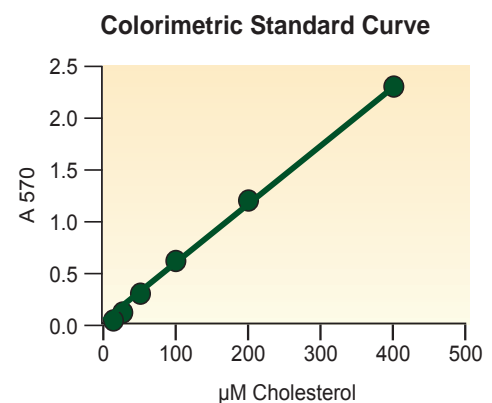
$\lambda_{\max}$  570 nm.  $\lambda_{\text{ex/em}}$  535/585 nm.

Colorimetric assay can be read on a spectrophotometer at 570 nm.

Fluorescence is measured at excitation 530 nm and emission 585 nm



*Fig.1 Cholesterol standard curve was generated using generated using fluorimetric detection: EX: 535 nm and Em 585 nm. out at 570 nm. R2 value=0.9977. Incubation time = 1 hour at Room Temp. Standard curve range 0.15625 μM to 10 μM.*



*Fig.2 Cholesterol standard curve colorimetric detection: absorbance read-out at 570 nm. R2 =0.9993. Incubation time=30 minutes. Standard curve range 1.25 μM to 80 μM.*

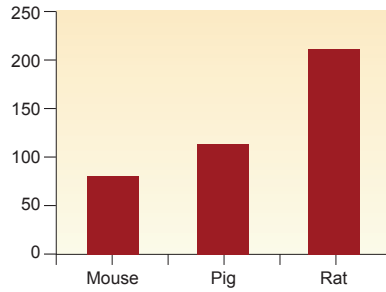


Fig.3 demonstrates the quantitation of total cholesterol in mg/dL in animal sera.

### Kit Contents

The Fluoro Cholesterol™ Kit contents include the following:

- Part #4022: Cholesterol Probe
- Part #6023: Enzyme Mix
- Part #3055: 1X Reaction Buffer
- Part #7018: Cholesterol Standard
- Part #7019: DMSO

### Ordering Information

The following Fluoro Cholesterol™ kit is available

Version/Catalog No.	Quantity	Price (in US dollars)
FLCHOL100-2	100 Tests	\$295

### References

Cholesterol and Triglyceride concentrations in serum/plasma pairs. Clin. Chem., 23/1, 60-63 (1977)

## Fluorescent Sarcosine Detection Kit - Fluoro Sarcosine™

### Key Benefits

The Key Benefits of our Fluoro Sarcosine™ Detection kit are as follows:

- Detection of sarcosine in cells or tissue extracts.
- Detection sarcosine in serum.
- Readout: 96 well Fluorescent Plate reader readout.

### Assay Principle

Sarcosine is natural amino acid that is an important intermediate in the metabolism of choline. Sarcosine is an important component of proteins and plays a significant role in metabolic processes of living cells as a source of as serine, creatine, purines and glutathione etc. Sarcosine is present in such food sources like legumes, eggs, turkey etc. It is used in a variety of industrial applications such as manufacturing of tooth paste and biodegradable surfactants. Sarcosine was recently implicated in the activation of prostate cancer cell and its detection as a possible marker for prostate cancer progression to metastasis (2) and as add additive treatment in Schizophrenia (3) and depression (4). Cell Technology's Fluoro Sarcosine™ assay provides a reliable, sensitive fluorimetric assay for the quantification of sarcosine in biological samples.

The Fluoro Sarcosine™ detection kit utilizes a non-fluorescent detection reagent, which is reduced via an enzyme-coupled reaction in the presence of sarcosine. A sarcosine standard curve is generated to quantify sarcosine in the samples.

### Reaction:

1. Sarcosine + non-fluorescent detection reagent + Enzyme Mix  $\longrightarrow$  fluorescent analog  
Excitation: 530-570 nm; Emission at 590-600 nm

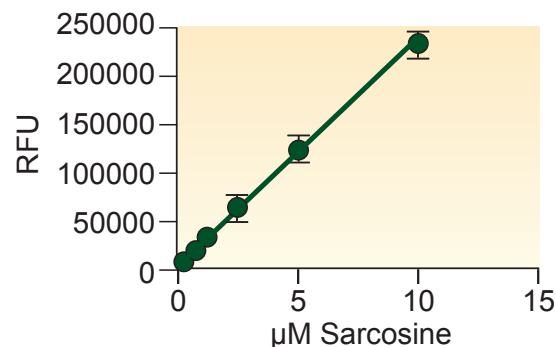


Figure 1. Sarcosine standard curve was generated as described in the protocol.  $R^2 = 0.998$

### Spike and recovery results:

Amount Sarcosine spiked in serum	% Recovery
75 μM	103
37.5 μM	104

Table 1: We conducted spike and recovery experiments to estimate % recovery of sarcosine. Serum was spiked with sarcosine with the concentrations mentioned in the table above. The samples were processed as described in section IX.

Amount Sarcosine spiked in cell lysates	% Recovery
75 $\mu$ M	97.03
37.5 $\mu$ M	98.80.

Table 2: We conducted spike and recovery experiments to estimate % recovery of sarcosine. Cell lysates were spiked with sarcosine with the concentrations mentioned in the table above. The samples were processed as described in section VIII: Mammalian Cell Preparation.

Sample	$\mu$ M Sarcosine
Serum A	1.529
Serum B	2.092
Serum C	2.486
Jurkats	1.461
Daudi	1.116

Table 3 Serum samples were diluted 1:5 in standard curve diluent. Jurkat and Daudi cells were prepared as described in the protocol. After the final wash cells were adjusted to  $1 \times 10^6$  cells/mL in standard curve diluents. Sarcosine was measured as described in the protocol.

## Kit Contents

The Fluoro Sarcosine™ Kit contents include the following:

- Part #6024: Enzyme Mix
- Part #3056: Standard Curve Diluent
- Part #4023: Sarcosine Detection Reagent
- Part #7021: Sarcosine Standard

## Ordering Information

The following Fluoro Sarcosine™ Kit is available

Catalog No.	Size	Price (in US dollars)
SARC 100-2	100	\$345

## References:

Wikipedia

- Sreekumar, Arun; Poisson, Laila M.; Rajendiran, Thekkelnaycke M.; Khan, Amjad P.; Cao, Qi; Yu, Jindan; Laxman, Bharathi; Mehra, Rohit et al. (2009). "Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression". *Nature* 457 (7231): 910. doi:10.1038/nature07762. PMID 19212411.
3. Lane H, Huang C, Wu P, Liu Y, Chang Y, Lin P, Chen P, Tsai G (2006). "Glycine transporter I inhibitor, N-methylglycine (sarcosine), added to clozapine for the treatment of schizophrenia". *Biol Psychiatry* 60 (6): 645–9. doi:10.1016/j.biopsych.2006.04.005. PMID 16780811.
4. <http://clinicaltrials.gov/ct2/show/NCT00977353> Clinicaltrials.gov "N-methylglycine (Sarcosine) Treatment for Depression"

# Section 6

## Section 6: Cytotoxicity Using Flow Cytometry

# Non Radioactive Assay for Cell / Antibody Mediated Cytotoxicity – ACT 1™

## Key Benefits

The Key Benefits of our the ACT1™ Assay for Cytotoxicity Kit are as follows:

- No radioactive materials required
- Works with a flow cytometer or fluorescence microscope
- Detects cytolytic activity at a cellular level
- Works with multiple types of mammalian cell lines

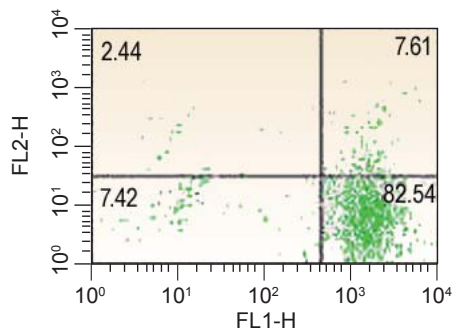
## Technology

The most commonly used method to measure CMC/ADCC is a radioactive chromium-51 ( $^{51}\text{Cr}$ ) release assays (2). There are several disadvantages with this assay: it is expensive, difficult to load certain cell types, expensive to dispose of due to strict environmental regulations, and has high background from spontaneous release of ( $^{51}\text{Cr}$ ). With the use of flow cytometry, it is now possible to eliminate the need for radioactive material and increase the ability to quantify cytolytic activity on a single cell bases. Various groups have demonstrated that measuring CMC/ADCC activity by flow cytometry has a strong (95%) correlation with the traditional ( $^{51}\text{Cr}$ ) release assay (3, 4, 5, 6).

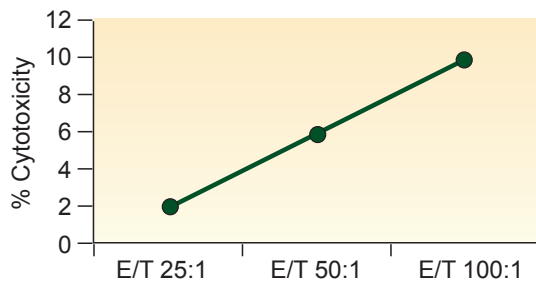
## Assay Principle

A cell tracking dye CFSE (7, 8, 9) is utilized to label the target cell population. After the assay has run its experimental protocol, 7AAD (live/dead) (10, 11) is added to measure cell death. 7AAD only enters membranecompromised cells and binds to DNA. Flow cytometry is utilized to gate on the target cells and measure 7AAD negative vs. 7AAD positive cells. % cytotoxicity is calculated by the following equation (see experimental example below):

7AAD positive (upper right quadrant) = R1 / 7AAD positive (upper right quadrant) + 7AAD negative (lower right quadrant) = R2 x100



(A) Figure A: To test natural killer ability of swine dg lymphocytes, K562 cells were stained and adjusted to a final concentration of  $1 \times 10^4$  cells/100 ul RPMI containing 10 % FBS. dg lymphocytes were added at E/T ratios of 25:1, 50:1, and 100:1 and adjusted to a total volume of 400 ul RPMI, then incubated for 4 hours at 37° C in a sterile capped facs tube. Following incubation live/dead stain was added directly to each tube, incubated for 15 min on ice and analyzed by flow cytometry.



(B) Figure B: % Cytotoxicity was determined using the formula  $(R1 / (R1 + R2)) \times 100$ . The data was plotted in a graph format

## Kit Contents

The ACT1™ Assay for Cytotoxicity Kit includes the following:

- Part #4002: Membrane stain, 4 vials of lyophilized powder
- Part #4003: Live/Dead stain, 3 vial containing of lyophilized powder
- Part #3003: 1 bottle 10X PBS with Ca<sup>2+</sup> and Mg<sup>2+</sup>

## Ordering Information

The following ACT1™ Assay for Cytotoxicity Kit is available:

Catalog No.	Quantity	Price (in US dollars)
ACT100-2	100+ Tests	\$275

## References

- a. Perussia, B., (1998). Current Topics in Microbiology and Immunology 230, p63. b. Whiteside, T.L., Rinaldo, C.R. and Herberman, R.B. (1992) Cytolytic Cellfunctions. In: N.R. Rose, E.C. de Macario (Eds.), Manual of Clinical Laboratory Immunology. American Society for Microbiology. Washington, DC, p. 220.
- Brunner, K.T., Manuel, J., Cerotini, J.C., Chapuis, B., (1968). Quantitative Assay of the Lytic Action of Immune Lymphoid Cells on Crlabelled Allogenic Target Cells in-vitro; Inhibition by Iso-antibody and by Drugs, Immunology 14,181.
- Lee-MacAry, A.E., Ross, E.L, Davies, D., and Wilkinson, R.W., (2001). Development of a Novel Flow Cytometric Cell-mediated Cytotoxicity Assay Using the Fluorophores PKH-26 and TO-PRO-3 Iodide. J. Immunology. Met 252, 83-92.
- Gogoy-Ramirez, K., Franck, K., and Gains, H., (2000). A Novel Method for the Simultaneous Assessment of Natural Killer Cell Conjugate Formation and Cytotoxicity at the Single-cell Level by Multi-parameter Flow Cytometry. Journal of Immunology. Met 239, 35-44.
- Goldberg, J.E., Sherwood, S.W., Clayberger, C., (1999). A Novel Method for Measuring CTL and NK Cell-mediated Cytotoxicity Using Annexin V and Two-color Flow Cytometry. Journal of Immunology. Methods 224, 1.
- Hatam, L, Schuval, S., Bonagura, V.R., (1994). Flow Cytometric Analysis of Natural Killer Cell Function as a Clinical Assay. Cytometry 16, 59.
- L.S De Clerck et al, J. Immunol. Meth. 172, 115 (1994).
- M. Bronner-Fraser, J. Cell Biol. 101, 610 (1985).
- M. Bronner-Fraser, J. Cell Biol. 101, 610 (1985).
- Rabinovitch, P.S., et al., J. Immunol. 136, 2769 (1986).
- Su, X., J. Immunol. 156, 156, 4198 (1996).
- Olin, MR. Lee, J. Choi, K, and Molitor, Tw.??? T-lymphocyte Cytotoxic Activity against Mycobacterium bovis Analysed by Flow Cytometry: Journal of Immunological Methods; Publication in process.
- Olin, MR. Thesis, K. Cho, J. and Molitor, T.W. Morphine Suppresses Microglial Directed Cytolytic Activity by ??? Lymphocytes. Journal of Neuroimmunology; Publication in process.



# Section 7

## Section 7: Oxidative Stress Detection

## Hydrogen Peroxide/Peroxidase Detection Kit – Fluoro H<sub>2</sub>O<sub>2</sub><sup>TM</sup>

### Key Benefits

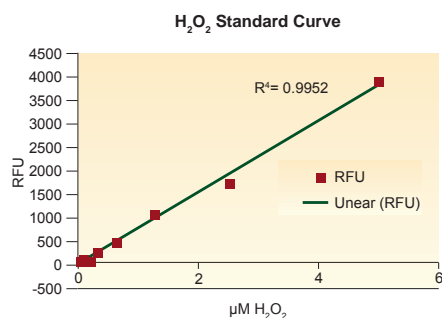
The Key Benefits of our the Fluoro H<sub>2</sub>O<sub>2</sub><sup>TM</sup> Kit are as follows:

- Non-Radioactive - Quick 10-minute assay
- Can monitor multiple time points to follow kinetics
- Dual mode, can detect H<sub>2</sub>O<sub>2</sub> or peroxidase activity
- One-step, no wash assay
- Adaptable for high throughput format
- Non-destructive cell based assay allows monitoring of additional parameter

### Assay Principle

The Fluoro H<sub>2</sub>O<sub>2</sub><sup>TM</sup> detection kit utilizes a non-fluorescent detection reagent to measure H<sub>2</sub>O<sub>2</sub>. H<sub>2</sub>O<sub>2</sub> oxidizes the detection reagent in a 1:1 stoichiometry to produce a fluorescent product resorufin. This oxidation is catalyzed by Peroxidase in a homogeneous no wash assay system.

The detection reagent can be utilized to detect H<sub>2</sub>O<sub>2</sub> release from cells or enzyme coupled reactions (1-7)



*In this figure, hydrogen peroxide was titrated in 1X reaction buffer and measured using the Fluoro H<sub>2</sub>O<sub>2</sub> Kit.*

### Reactivity

H<sub>2</sub>O<sub>2</sub> + Detection Reagent (non-fluorescent) Peroxidase  $\longrightarrow$  Resorufin (fluorescent)  
Excitation: 530-571 nm; Emission: 590-600 nm

### Kit Contents

The Fluoro H<sub>2</sub>O<sub>2</sub><sup>TM</sup> Kit includes the following:

- Part #3011: 5X Reaction Buffer: 20 ml of 0.25M sodium phosphate buffer, pH 7.4
- Part #4007: Detection reagent: Five vials, each vial for 100 assays.
- Part #3012: Hydrogen Peroxide: 200µL of a stabilized 3% solution
- Part #6004: Horseradish Peroxidase: 18.9 Units of enzyme

## Ordering Information

The following Fluoro H<sub>2</sub>O<sub>2</sub><sup>TM</sup> Kit is available:

Catalog No.	Quantity	Price (in US dollars)
FLOH100-3	500 Tests	\$225

## References

Anal Biochem 253, 162 (1997);

J. Immunol Methods 202, 133 (1997);

J Neurochem 79, 266 (2001);

Am J Physiol Lung Cell Mol Physiol 281, L993 (2001);

Mol Hum Reprod 7, 237 (2001);

Anal Biochem 287, 196 (2000);

## Nitric Oxide Detection Kit – NOS Detection

### Key Benefits

The Key Benefits of our NOS Detection Kit are as follows:

- Cell permeable
- No wash homogenous assay.
- Adaptable to High throughput assay platforms
- Real-time detection of NOS activity

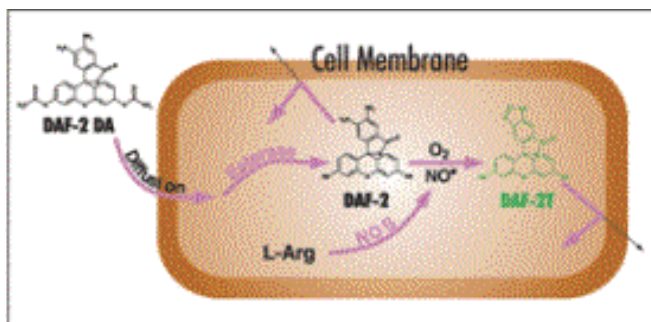
### Assay Principle

Diaminofluorescein –2 Diacetate (DAF-2DA) is a non-fluorescent cell permeable reagent that can measure free Nitric Oxide (NO) and nitric oxide synthase (NOS) activity in living cells under physiological conditions. Once inside the cell the diacetate groups on the DAF-2DA reagent are hydrolyzed by cytosolic esterases thus releasing FAD-2 and sequestering the reagent inside the cell. Production of nitric oxide converts the non-fluorescent dye, DAF-2, to its fluorescent triazole derivative, DAF-2T.

DAF-2T can be observed by: excitation 488 nm and measuring emission at 515 nm.

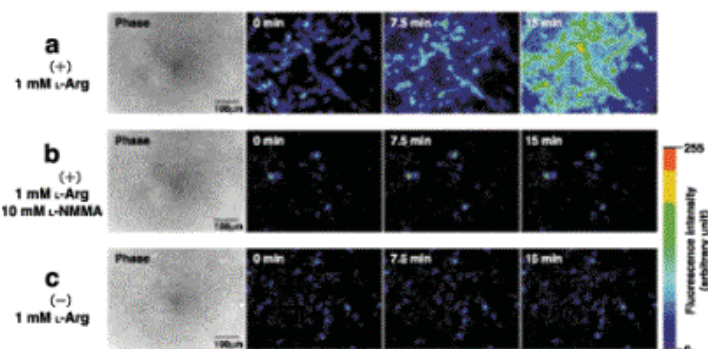
### Reactivity

DAF-2DA can be used to detect NOS activity in cell culture or tissue sections. This reagent is not species specific and can also be used to detect NOS activity in plant cells.



(A)

Figure A: DAF-2DA, the nonfluorescent probe, enters the cells where cytosolic esterases hydrolyze the diacetate groups, releasing the nonfluorescent DAF-2 dye. DAF-2 then reacts with NO and O<sub>2</sub> to produce DAF-2T, its triazole fluorescent derivative.



(B)

Fig B. Phase Contrast and fluorescent microscopic images of rat aorta-derived vascular smooth muscular cells loaded with DAF-2DA.

### Production Specifications

Formula: C<sub>24</sub>H<sub>18</sub>N<sub>2</sub>O<sub>7</sub>; MW: 446.4; Appearance: Liquid  
 Formulation: DAF-2DA is dissolved in DMSO at approx 5 mM.  
 Manufactured: by Daiichi Pure Chemicals Co. Ltd. Japan

## Kit Contents

The NOS Detection Kit includes the following:

- Part #4016: DAF - 2DA

## Ordering Information

The following NOS Detection Kits are available

Catalog No.	Quantity	Price (in US dollars)
NOS200-1	0.125 mg per vial at 2.22 mg/mL.	\$145
NOS200-2	0.25 mg per vial at 2.22 mg/mL.	\$290

**NOTE:** Volume discounts are available. Please call for pricing.

## References

Direct evidence of NO production in rat hippocampus and cortex using a new fluorescent indicator: DAF-2 DA: H. Kojima, et al.; *Neuroreport* 9, 3345 (1998) Abstract

Direct evidence of nitric oxide production from bovine aortic endothelial cells using new fluorescence indicators: diaminofluoresceins: N. Nakatsubo, et al; *FEBS Lett.* 427, 263 (1998) Abstract

Nitric Oxide Ameliorates Hydrophobic Bile Acid-induced Apoptosis in Isolated Rat Hepatocytes by Non-mitochondrial Pathways: E. Gumprich, et al.; *J. Biol. Chem.* 277, 25823 (2002) Full Text; <http://www.jbc.org/cgi/content/full/277/28/25823#SEC1>

Development of a fluorescent indicator for nitric oxide based on the fluorescein chromophore: H. Kojima, et al.; *Chem. Pharm. Bull.* 46, 373 (1998) Abstract

Detection and imaging of nitric oxide with novel fluorescent indicators: diaminofluoresceins: H. Kojima, et al.; *Anal. Chem.* 70, 2446 (1998) Abstract

## Fluorescent Hypochlorite (OCI-) Detection Kit

### Key Benefits

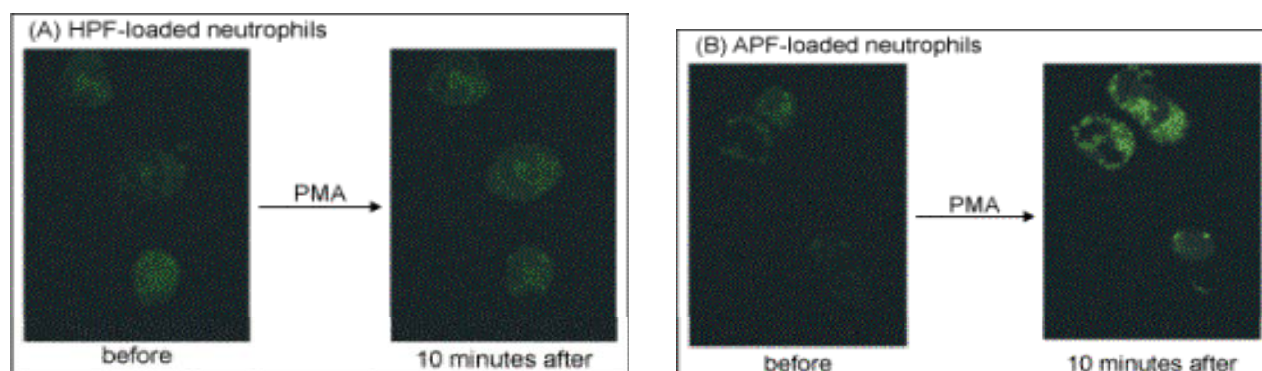
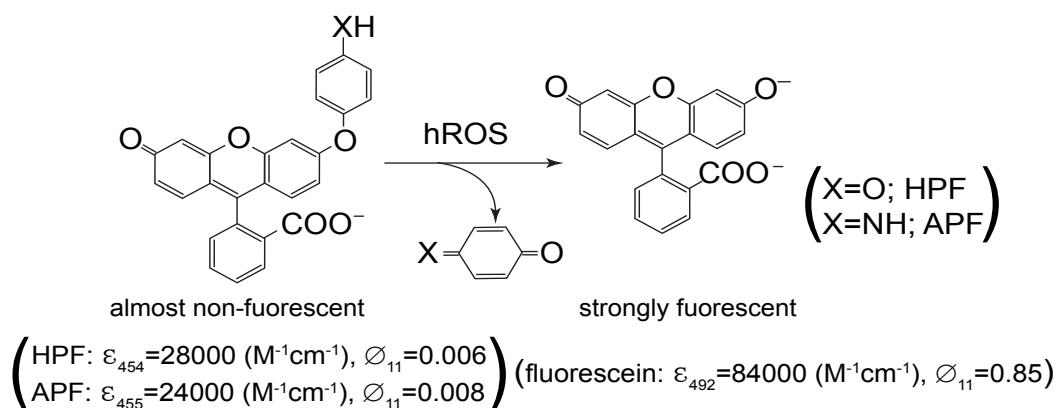
The Key Benefits of our Fluorescent OCI Detection Kit are as follows:

- Quenched Cell permeable dyes
- One Step, No wash Homogenous assay.
- Adaptable to High throughput assay platforms
- Can monitor multiple time points to follow real time kinetics
- Non-destructive cell based assay allows monitoring of additional parameters

### Introduction

The two new novel probes, Aminophenyl Fluorescein (APF) and Hydroxyphenyl Fluorescein (HPF) developed by Tetsuo Nagano et. al. <sup>(1)</sup>, are selective for the detection of highly reactive oxygen species (hROS). Both probes have little reactivity towards other ROS such as: singlet oxygen ( $O_2^1$ ), superoxide ( $O_2^{\bullet-}$ ), hydrogen peroxide ( $H_2O_2$ ), nitric oxide ( $NO\bullet$ ), and alkyl peroxide ( $RO_2\bullet$ ) (see table below) <sup>(1)</sup>. HPF/APF are cell permeable and can be used in combination to detect hypochlorite ( $-OCI$ ) production in cells (see fig 1). Hypochlorite can be detected by loading two samples, one with APF and the other with HPF. Hypochlorite production is visualized by increase in fluorescence of APF loaded cells and no increase in fluorescence in HPF loaded cells.

### Assay Principle



Above: Detection of Hypochlorite ( $-OCI$ ) in neutrophils. Neutrophils were isolated from porcine blood, washed in Krebs-Ringers phosphate buffer (as described in step V 1. above) and seeded in glass bottom dishes. The cells were then loaded with APF or HPF (10mM final) by incubation for 30 minutes at room temperature. The Dye-loaded neutrophils were stimulated with PMA (2 ng/mL). Fluorescence images were acquired before and 10 minutes after stimulation. Excitation: 488 nm emission: 505-550 nm barrier filters 1. Hypochlorite production can be detected with increase APF fluorescence

and no increase in HPF fluorescence.

## Reactivity

The table below represents the reactivity profile of APF/HPF:

ROS (RFU)	HPF (RFU) Ex: 499 nm Em: 515 nm	APF (RFU) Ex: 499 nm Em: 515 nm	DCFH-DA (RFU) Ex: 500 nm Em: 520 nm
Hydroxyl Radical: .OH	730	1200	7400
Peroxynitrite: ONOO	120	560	6600
Hypochlorite: -OCl	6	3600	86
Oxygen Radical: $1O_2$	5	9	26
Superoxide: $O_2^-$	8	6	67
Hydrogen Peroxide: $H_2O_2$	2	<1	190
Nitric Oxide: NO	6	<1	150
Alkylperoxyl Radical: ROO.	17	2	710
Autooxidation	<1	<1	2000

## Kit Contents

The Fluorescent OCL Detection Kit includes the following:

- Part #4011: APF, 1 vial
- Part #4012: HPF, 1 vial

## Ordering Information

The following Fluorescent OCl Detection Kit is available:

Catalog No.	Quantity	Price (in US dollars)
FLOCL100-2	150 Tests*	\$445

\* Test size depends on dilution and volume used per test. For example in a test volume of 0.2 mL:

1:500 (10  $\mu$ M final) dilution = 147 tests

1:1000 (5  $\mu$ M final) dilution = 294 tests

## References

Ken-ichi Setsukinai, Yasuteru Urano, Katsuko Kakinuma, Hideyuki J. Majima, and Tetsuo Nagano. Development of Novel Fluorescence Probes That Can Reliably Detect Reactive Oxygen Species and Distinguish Specific Species. THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 278, No. 5, Issue of January 31, pp. 3170–3175, 2003

Manufactured by Daiichi Pure Chemicals Co. Ltd. Japan

## Fluorescent Hydroxyl (OH) / Peroxynitrite (ONOO-) Detection Kit

### Key Benefits

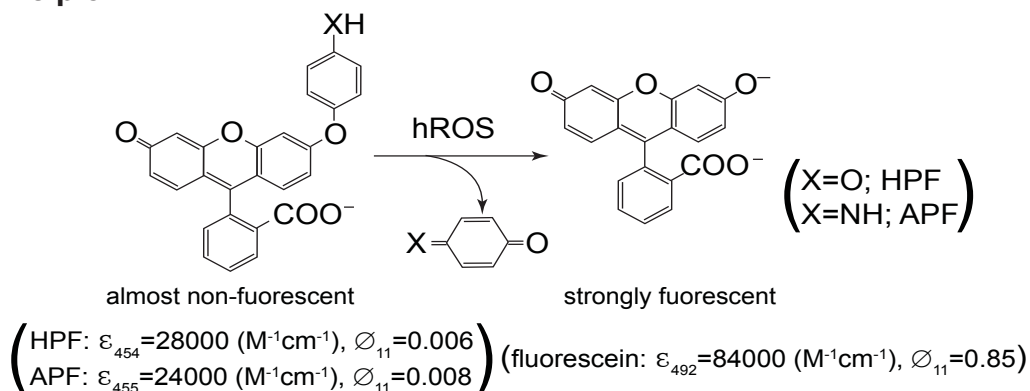
The Key Benefits of our the OH/ONOO- Detection Kit are as follows:

- Quenched cell permeable dye
- One step, no wash homogenous assay.
- Adaptable to high throughput assay platforms
- Can monitor multiple time points to follow real time kinetics
- Non-destructive cell based assay allows monitoring of additional parameters

### Introduction

A new novel probe, hydroxyphenyl fluorescein (HPF), developed by Tetsuo Nagano et. al. <sup>(1)</sup>, is a highly selective probe for the detection of highly Reactive Oxygen Species (hROS). It is a cell permeable highly sensitive fluorescent probe for hydroxyl radical (OH•), and peroxynitrite (ONOO-) detection. It has little reactivity towards other hROS such as: hypochlorite (-OCl), singlet oxygen (O<sub>2</sub><sup>1</sup>), superoxide (O<sub>2</sub><sup>-•</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), nitric oxide (NO•), and alkyl peroxide (RO<sub>2</sub>•) (see table below) <sup>(1)</sup>.

### Assay Principle



### Reactivity

The table below represents the reactivity profile of HPF:

ROS (RFU)	HPF (RFU) Ex: 499 nm Em: 515 nm	DCFH-DA (RFU) Ex: 500 nm Em: 520 nm
Hydroxyl Radical: .OH	730	7400
Peroxynitrite: ONOO	120.	6600
Hypochlorite: -OCl	6	86
Oxygen Radical: 1O <sub>2</sub>	5	26
Superoxide: O <sub>2</sub>	8	67
Hydrogen Peroxide: H <sub>2</sub> O <sub>2</sub>	2	190
Nitric Oxide: NO	6	150

ROS (RFU)	HPF (RFU) Ex: 499 Em: 515	DCFH-DA (RFU) Ex: 500 Em: 520
Alkylperoxyl Radical: ROO	17	710
Autooxidation	<1	2000

### Kit Contents

- Part #4012: HPF, 1 vial

### Ordering Information

The following OH/ONOO- Detection Kit is available:

Catalog No.	Quantity	Price (in US dollars)
FLHPF100-2	150 Tests*	\$225

\* Test size depends on dilution and volume used per test. For example in a test volume of 0.2 mL:

1:500 (10  $\mu$ M final) dilution = 147 tests

1:1000 (5  $\mu$ M final) dilution = 294 tests

**NOTE:** Volume discounts are available. Please call for pricing.

### References

Ken-ichi Setsukinai, Yasuteru Urano, Katsuko Kakinuma, Hideyuki J. Majima, and Tetsuo Nagano. Development of Novel Fluorescence Probes That Can Reliably Detect Reactive Oxygen Species and Distinguish Specific Species. THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 278, No. 5, Issue of January 31, pp. 3170–3175, 2003

Manufactured: by Daiichi Pure Chemicals Co. Ltd. Japan

## Fluorescent hROS Detection Kit

### Key Benefits

The Key Benefits of our Fluorescent hROS Detection Kit are as follows:

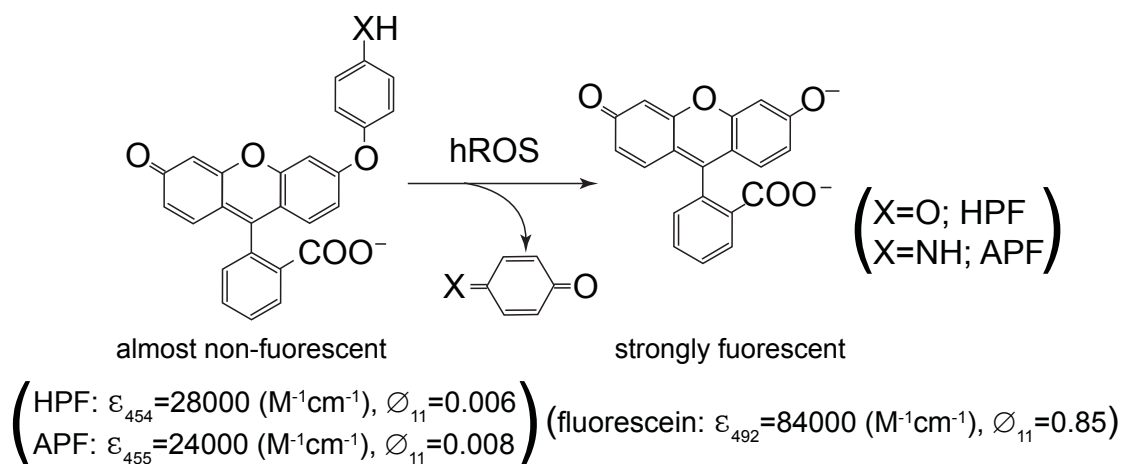
- Quenched cell permeable dye
- One step, no wash homogenous assay.
- Adaptable to high throughput assay platforms
- Can monitor multiple time points to follow real time kinetics
- Non-destructive cell based assay allows monitoring of additional parameters
- Readout – Plate Reader, fluorescent microscope, flow cytometry

### Introduction

A new novel probe, Aminophenyl fluorescein (APF), developed by Tetsuo Nagano et. al. (1), is a general selective indicator for the detection of highly Reactive Oxygen Species (hROS). The probe has little reactivity towards other ROS such as: singlet oxygen ( $O_2^1$ ), superoxide ( $O_2^{\bullet-}$ ), hydrogen peroxide ( $H_2O_2$ ), nitric oxide ( $NO^{\bullet}$ ), and alkyl peroxide ( $RO_2^{\bullet}$ ) (see table below)1. APF is a cell permeable indicator that can be used to detect Hydroxyl Radical ( $\cdot OH$ ), Peroxynitrite: ( $ONOO^-$ ) and hypochlorite ( $-OCl$ ) production in cells.

Several fluorescent probes such as 2',7'-dichlorodihydrofluorescein (DCFH) and di-hydrorhodamine 123, have been developed to detect Reactive Oxygen Species (ROS). As Hempel and co-workers (2) have indicated, DCFH and dihydrorhodamine 123 can react with various ROS and oxidizing species such as hydrogen peroxide, hypochlorite, superoxide, nitric oxide, ferrous ion, and others. These probes should be considered as detecting a broad range of ROS (2).

### Assay Principle



### Reactivity

The table below represents the reactivity profile of APF:

ROS (RFU)	APF (RFU) Ex: 499 nm Em: 515 nm	DCFH-DA (RFU) Ex: 500 nm Em: 520 nm
Hydroxyl Radical: $\cdot OH$	1200	7400
Peroxynitrite: $ONOO^-$	560	6600
Hypochlorite $-OCl$ :	3600	86

ROS (RFU)	APF (RFU) Ex: 499 nm Em: 515 nm	DCFH-DA (RFU) Ex: 500 nm Em: 520 nm
Oxygen Radical: $1O_2$	9	26
Superoxide: $O_2^-$	6	67
Hydrogen Peroxide: $H_2O_2$	<1	190
Nitric Oxide: NO	<1	150
Alkylperoxyl Radical: ROO	2	710
Autooxidation	<1	2000

### Kit Contents

- Part #4011: APF, 1 vial

### Ordering Information

The following OH/ONOO- Detection Kit is available

:

Catalog No.	Quantity	Price (in US dollars)
FLAPF100-2	150 Tests*	\$225

\*Test size depends on dilution and volume used per test. For example in a test volume of 0.2 mL:

1:500 (10  $\mu$ M final) dilution = 147 tests

1:1000 (5  $\mu$ M final) dilution = 294 tests

**NOTE:** Volume discounts are available. Please call for pricing.

### References

Ken-ichi Setsukinai, Yasuteru Urano, Katsuko Kakinuma, Hideyuki J. Majima, and Tetsuo Nagano. Development of Novel Fluorescence Probes That Can Reliably Detect Reactive Oxygen Species and Distinguish Specific Species. THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 278, No. 5, Issue of January 31, pp. 3170–3175, 2003

Manufactured: by Daiichi Pure Chemicals Co. Ltd. Japan

## Colorimetric Super Oxide Dismutase Detection Kit - SOD

### Key Benefits

The Key Benefits of our SOD Detection Kit are as follows:

- 100% Inhibition by Super Oxide Dismutase (SOD)
- Can detect low concentrations of SOD
- Highly water-soluble formazan dye.
- Applications: colorimetric detection
- Readout: colorimetric

### Introduction

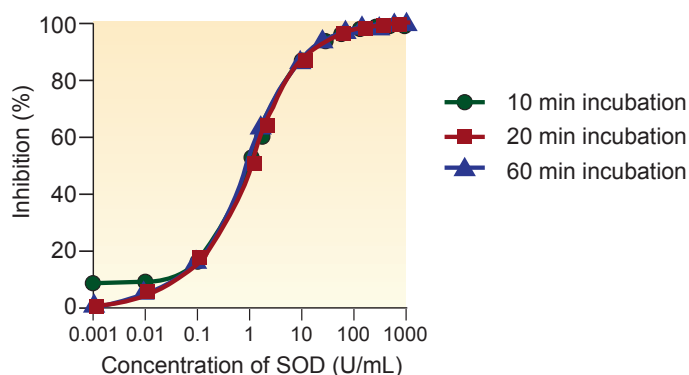
Superoxide dismutase (SOD) are metalloenzymes that catalyze the dismutation of superoxide radical into hydrogen peroxide ( $H_2O_2$ ) + molecular oxygen ( $O_2$ ) and consequently provide an important defense mechanism against superoxide radical toxicity (1).

Oxidative stress dependent upon superoxide radical can account for a number of acute and chronic disease states, which include inflammation and ischemia-reperfusion (2, 3). SOD protects murine peritoneal macrophages from apoptosis induced by adriamycin (4). Furthermore, overexpression of SOD in fibrosarcoma cells, protects against apoptosis and promotes cell differentiation (5).

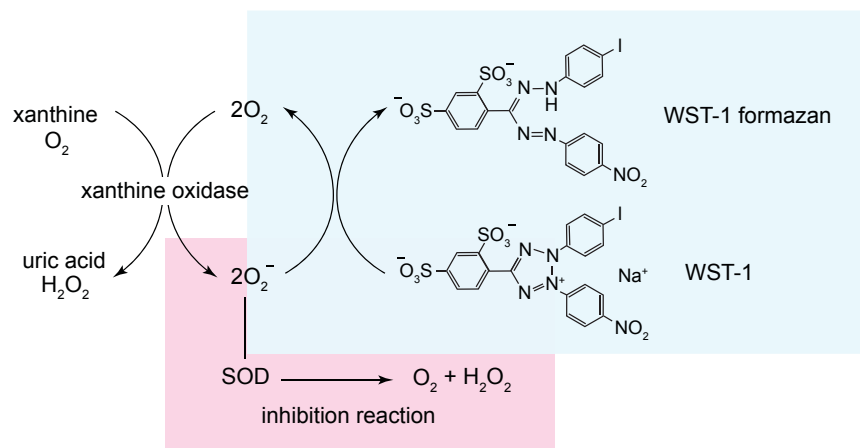
### Assay Principle

To determine SOD activity, several direct and indirect methods have been developed. A common and convenient indirect method utilizes nitroblue tetrazolium (NBT) conversion to NBT-diformazan (formazan dye) via superoxide radical. However, there are several disadvantages to the NBT method, such as poor water solubility of the formazan dye and the interaction with the reduced form of xanthine oxidase. Though cytochrome C is also commonly used for SOD activity detection, its reactivity with superoxide is too high to determine low levels of SOD activity.

Cell Technology's SOD kit utilizes a water-soluble tetrazolium salt, WST-1 (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) that produces a highly water-soluble formazan dye upon reduction with a superoxide anion (6). The rate of the reduction with  $O_2^-$  is linearly related to the xanthine oxidase (XO) activity, and is inhibited by SOD, as shown in Figure A. Therefore, the IC<sub>50</sub> (50% inhibition activity of SOD or SOD-like materials) can be determined by this colorimetric method. Absorbance can be measured at 440nm.



(A) Figure A: Inhibition curve prepared using SOD from bovine liver



(B) Figure B: SOD ASSAY reaction

### Kit Contents

The SOD Detection Kit includes the following:

- Part # 4014: 20X WST-1 solution: 1 ml
- Part # 6010: Xanthine oxidase solution (XO): 15  $\mu$ L
- Part # 3029: Assay buffer: 20 mL.
- Part # 3030: Xanthine oxidase dilution buffer: 10mL. (XO dilution buffer)
- Part # 6011: SOD Enzyme: 30  $\mu$ L; see vial for activity.
- Part # 9001: 96-well ELISA plate: 1 plate
- Part # 9002: Adhesive plate cover: 2

### Ordering Information

The following SOD Detection Kit is available:

Catalog No.	Quantity	Price (in US dollars)
CSOD100-2	100 Tests	\$225

**NOTE:** Volume discounts are available. Please call for pricing.

### References

- Malstrom, B., Andreasson, L., and Reinhammer, B. in *The Enzymes*. Byer, P., editor. XIIB, Academic Press, New York (1975).
- Lontz, W., Sirsjo, J., Liu, W., Lindberg, M., Rollman, O., and G. (1976) *Int. J. Cancer* 17, 62–70. Torma, H. (1995) *Free Radical Biol. Med.* 18, 349–355.
- Janero, D. R. (1995) *CRC Crit. Revs. Food Sci. Nutr.* 35, 65–81
- Dominguez-Rodriguez, J.R. et al. (2001) *Anticancer Res.* 21:1869.
- Zhao, Y. et al. (2001) *Antioxid. Redox Signal* 3:375.
- H. Ukeda, A. K. Sarker, D. Kawana and M. Sawamura, *Anal. Sci.*, 15, 353 (1999).

## Fluorescent Glutathione S-Transferase Activity Kit - Fluoro GST™

### Key Benefits

The Key Benefits of our Fluoro GST™ Detection Kit are as follows:

- Sensitive – measures < 100 U of GST Activity
- Convenient – stable, 4 °C liquid reagents
- Flexible – can be used for kinetic or end point measurement
- Selective – limited interference from solvents, detergents or bilirubin
- Can detect Glutathione S-Transferase activity in biological samples such as serum, plasma, urine and cell lysates.

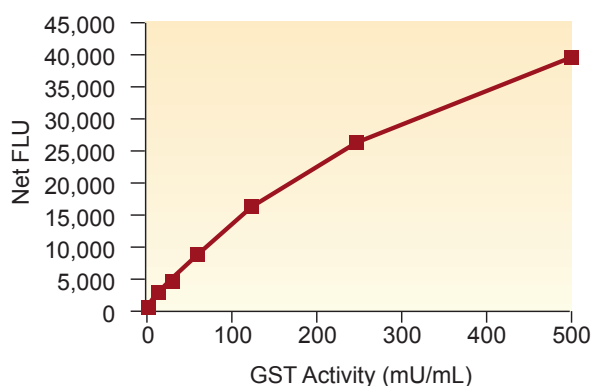
### Introduction

The Glutathione S-Transferase (GST) family of isozymes function to detoxify and neutralize a wide variety of electrophilic molecules by mediating their conjugation with reduced glutathione <sup>(1)</sup>. Human GSTs are encoded by 5 gene families, expressing in almost all tissues as four cytosolic and one microsomal forms. Dividing the family by isoelectric points, the basic alpha (pI 8–11), the neutral mu (pI 5–7) and acidic pi (pH<5) classes are populated by additional subclasses, each isozyme displaying differential specificity for given electrophilic molecules <sup>(2)</sup>.

Given its pivotal role in ameliorating oxidative stress/damage, GST activity has been repeatedly investigated as a biomarker for arthritis, asthma, COPD, and multiple forms of cancer, as well as an environmental marker <sup>(3-7)</sup>. Examination of GST isoforms and activity in human cancers, tumors and tumor cell lines has revealed the predominance of the acidic pi class. Furthermore, this activity is thought to substantially contribute to the innate or acquired resistance of specific neoplasms to anticancer therapy <sup>(8,9)</sup>.

The Fluoro GST™ kit is designed to quantitatively measure the activity of GST present in a variety of samples. Please read the complete kit insert before performing this assay. A GST standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. The kit utilizes a non-fluorescent molecule that is a substrate for the GST enzyme that covalently attaches to glutathione (GSH) to yield a highly fluorescent product. Mixing the sample or standard with the supplied Detection Reagent and GSH and incubating at room temperature for 30 minutes yields a fluorescent product that is read at 460 nm in a fluorescent plate reader with excitation at 390 nm. The activity of the GST in the sample is calculated, after making a suitable correction for any dilution of the sample, using software available with most fluorescence plate readers.

### Typical Standard Curve



**Always run your own standard curve for calculation of results.  
Do not use this data.**

## Kit Contents

The Fluoro GST™ Detection Kit includes the following:

- Part # 90036: Black Half Area Well Plate
- Part # 90037: GST Detection Reagent
- Part # 90038: Dry DMSO
- Part # 90039: Assay Buffer
- Part # 90040: Glutathione
- Part # 90041: Glutathione S-Transferase Standard

## Ordering Information

The following Fluoro GST™ Detection Kit is available:

Catalog No.	Size	Price (in US dollars)
GSTR-100	100 Tests	\$345

## References:

1Habig, W, et al. "Glutathion S-Transferases: The First Enzymatic Step in Mercapturic Acid Formation" J. Biol.Chem. 1974 249(22):7130-7139.

2Cook, JA, et al., "Differential Specificity of Monochlorobimane for Isozymes of Human and Rodent Glutathione S-Transferases" Cancer Res. 1991 51:1606-1612.

3Dalle-Donne, I, et al. "Biomarkers of Oxidative Damage in Human Disease" Clinical Chemistry, 2006 52(4):601-623.

Surapneni, KM & VSC Gopan, "Lipid Peroxidation and Antioxidant Status in Patients with Rheumatoid Arthritis" Ind.J.Clin.Biochem. 2008 23(1):41.44.

Mohan, SK & O Venkataramana. "Status of Lipid Peroxidation, Glutathione, Ascorbic Acid, Vitamin E and Antioxidant Enzymes in Patients with Osteoarthritis" Ind.J.Med. Sci." 2007 61:9-14.

Ferrandina, G., et al., "Glutathione S-Transferase Activity in Epithelial Ovarian Cancer: Association with Response to Chemotherapy and Disease Outcome" Ann.Oncol. 1997 8:343-350.

Otitoju, O & Onwarah, INE. "Glutathione S-transferase (GST) Activity as a Biomarker in Ecological Risk Assessment of Pesticide Contaminated Environment" African J. Biotech. 2007 6(12)1455-1459.

Shea, TC, et al., "Identification of an Anionic Form of Glutathione Transferase Present in Many Human Tumors and Human Tumor Cell Lines" Cancer Res. 1988 48:527-533.

Shea, TC, et al., "Glutathione Transferase Activity and Isozyme Composition in Primary Human Breast Cancers" Cancer Res. 1990 50:6848-6853.



# Section 8

## Section 8: Ultra pure chemicals

## Ultra Pure Grade Adenosine 5'-diphosphate – Ultra Pure ADP

### Purity

Greater than 99.9% pure as tested by P31 NMR.

Molecular weight: 427.20

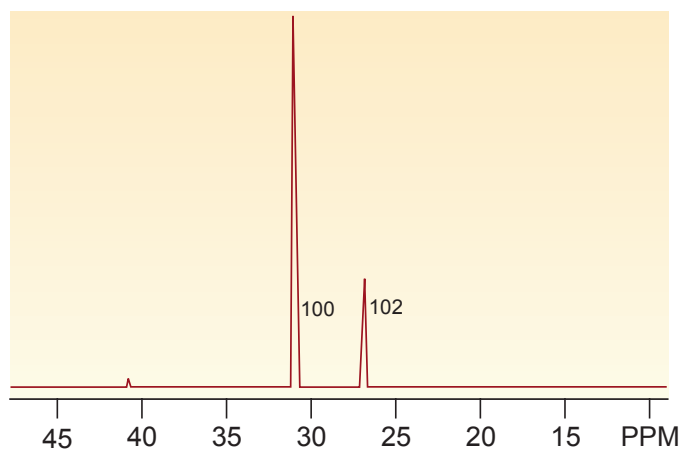
Molecular formula:  $C_{10}H_{15}N_5O_{10}P_2$

### Introduction

Commercially available ADP is contaminated with trace amounts of ATP. In bioluminescence assays this trace ATP contamination results in high backgrounds and reduces assay sensitivity. Cell Technology's ADP is purified to remove these trace ATP contaminants and as a result produces ADP that is greater than 99.9% pure.

### Application

This ADP is designed for use in bioluminescence applications where minimal background ATP contamination is required. ADP can be used as a substrate in enzymatic reactions to generate ATP. Subsequently ATP can be detected via the bioluminescence reaction of luciferase/luciferin.



### Kit Contents

Supplied as a 10 mM solution buffered in 60mM TRIS at pH 7.0.

### Ordering Information

The following Ultra Pure ADP kits are available

Catalog No.	Quantity	Price (in US dollars)
ADP100-2	1 mL	\$225
ADP100-3	5 mL	\$945

### Precautions and Storage:

Avoid repeated freeze/thaw cycles by freezing down as aliquots.

Product should be stored  $\leq -20^{\circ}\text{C}$



# Section 9

## **Section 9: Coating Buffers and ELISA Diluents**

## Block Buffers

### Key Benefits

Block Buffers improve assays in the following ways:

- Increase the sensitivity of the assay – This is done by stabilizing the antigenic and functional regions of the absorbed protein.
- Reduce background noise - Block buffers inhibit nonspecific binding to uncoated regions of the plate and non-specific binding sections of the absorbed proteins. This leads to lower background noise and high specific signal.
- Increase efficiency and extending the shelf-life of the coated plates - With a longer shelf-life, plates may be prepared in large batches to be used over time. Large batch sizes will increase efficiency and reduce plate-to-plate variability to increase consistency.
- Conserve valuable reagents - By promoting a higher specific signal, less protein may be needed to coat the plate and less of the detection molecules may be needed to generate a signal when the proper blocker is used.

### Availability

The following Block Buffers are available:

#### EBB 100 General-Purpose Blocker with BSA-Based Blockers

Catalog No	Quantity (mL)	# Plates at 300 $\mu$ L/well (28.8 mL/plate)	# Plates at 400 $\mu$ L/well (38.4 mL/plate)	Price (in US dollars)
EBB100-1	100 Tests	3	2	\$45
EBB100-2	500 Tests	16	12	\$120
EBB100-3	1,000 Tests	33	25	\$199
EBB100-4	10,000 Tests	330	250	\$1,900

#### EBB 200 Non-Mammalian-Based Blockers

Catalog No	Quantity (mL)	# Plates at 300 $\mu$ L/well (28.8 mL/plate)	# Plates at 400 $\mu$ L/well (38.4 mL/plate)	Price (in US dollars)
EBB200-1	100 Tests	3	2	\$45
EBB200-2	500 Tests	16	12	\$140
EBB200-3	1,000 Tests	33	25	\$270
EBB200-4	10,000 Tests	330	250	\$2,500

#### EBB 300 Synthetic Blocker with Tween

Catalog No	Quantity (mL)	# Plates at 300 $\mu$ L/well (28.8 mL/plate)	# Plates at 400 $\mu$ L/well (38.4 mL/plate)	Price (in US dollars)
EBB300-1	100 Tests	3	2	\$45
EBB300-2	500 Tests	16	12	\$170
EBB300-3	1,000 Tests	33	25	\$270
EBB300-4	10,000 Tests	330	250	\$2,300

## Assay Diluents

### Key Benefits

Assay Diluents improve assays in the following ways:

- Reduce the variations among samples and diluents used to generate standard curves  
Reduce background noise caused by non-specific interactions between the sample matrix proteins and the plate surface

### Availability

The following Assay Diluents are available:

#### EAD 100 General Assay Diluent for Serum and Plasma Samples (Sandwich Format)

Catalog No	Quantity (mL)	# Plates at 50 $\mu$ L/well (4.8 mL/plate)	# Plates at 100 $\mu$ L/well (9.6 mL/plate)	Price (in US dollars)
EAD100-1	100 Tests	20	10	\$55
EAD100-2	500 Tests	101	51	\$195
EAD100-3	1,000 Tests	205	103	\$295
EAD100-4	10,000 Tests	2,075	1,030	\$2,400

#### EAD 200 IgM (Positive Interference) Reducing Assay Diluent (all ELISAs)

Catalog No	Quantity (mL)	# Plates at 50 $\mu$ L/well (4.8 mL/plate)	# Plates at 100 $\mu$ L/well (9.6 mL/plate)	Price (in US dollars)
EAD200-1	100 Tests	20	10	\$75
EAD200-2	500 Tests	101	51	\$230
EAD200-3	1,000 Tests	205	103	\$395
EAD200-4	10,000 Tests	2,075	1,030	\$3,195

#### EAD 300 Non-Mammalian-Based Assay Diluent (Ag Down and Sandwich ELISAs)

Catalog No	Quantity (mL)	# Plates at 50 $\mu$ L/well (4.8 mL/plate)	# Plates at 100 $\mu$ L/well (9.6 mL/plate)	Price (in US dollars)
EAD300-1	100 Tests	20	10	\$75
EAD300-2	500 Tests	101	51	\$230
EAD300-3	1,000 Tests	205	103	\$395
EAD300-4	10,000 Tests	2,075	1,030	\$3,195

#### EAD 400 Antigen-Down Assay Diluent (Ag Down and Sandwich ELISAs)

Catalog No	Quantity (mL)	# Plates at 50 $\mu$ L/well (4.8 mL/plate)	# Plates at 100 $\mu$ L/well (9.6 mL/plate)	Price (in US dollars)
EAD400-1	100 Tests	20	10	\$75
EAD400-2	500 Tests	101	51	\$230
EAD400-3	1,000 Tests	205	103	\$395
EAD400-4	10,000 Tests	2,075	1,030	\$3,195

## Universal Plate Coating Buffers

### Key Benefits

Universal Plate Coating Buffers improve assays in the following ways:

- Save valuable reagents. By promoting a higher specific signal, less protein may be needed to coat the plate and less of the detection molecules may be needed to generate a signal when this buffer is used.
- Increase the shelf-life of your plates. Depending on the activity of the coated protein, plates may be stored at room temperature or 2-8°C for several months, or even years under proper conditions.
- Increase reproducibility. By using consistent reagents from a reliable source, your ELISAs may become more reproducible.
- Increase the signal of the ELISA. If the antibody or antigen is not sticking to the plate, or unwanted proteins are interfering with binding, the plate will have an overall low specific signal. CB1 may foster adsorption and reduce interference.
- Decrease background noise. If excess proteins are sticking to the plate, plate will have overall high backgrounds (a high signal overall, but a low specific signal). Universal Plate Coating Buffer may reduce interference.

### Availability

The following Universal Plate Coating Buffers are available:

Catalog No.	5x Bottle Size	# Plates at 50 $\mu$ L/well (4.8 mL/plate)	# Plates at 200 $\mu$ L/well (9.6 mL/plate)	# Plates at 300 $\mu$ L/well (28.8 mL/plate)	Price/Bottle (in US dollars)
ECB100-1	100 mL	100	25	16	\$40
ECB100-2	500 mL	510	125	84	\$125
ECB100-3	1 L	1,020	250	170	\$195
ECB100-4	10 L	10,200	2,500	1,700	\$1,350

## Conjugate Diluents

### Key Benefits

Conjugate Diluents Improve Assays in the following ways:

- Increase the sensitivity of the assay
- Decrease backgrounds
- Stabilize newly conjugated proteins; also reconstitute lyophilized conjugates and diluents concentrates conjugates
- Prolong the shelf-life of HRP-conjugated proteins and enhance their utility in ELISA applications
- Extend the useful titer of the conjugate
- Conserve valuable reagents; by promoting a higher specific signal, less conjugate may be needed to generate a signal when a stabilizing conjugate diluent is used.
- Lengthen the shelf-life of conjugated antibodies for future use. With a longer shelf-life, conjugates may be prepared in batches for use in future plate coating runs. Using conjugates from the lot generally reduces inter-assay variability and increases consistency.

### Availability

The following Conjugate Diluents are available:

#### ECD 100 Monoclonal-Polyclonal Conjugate Diluent and Stabilizer

5x Monoclonal – Polyclonal Diluent and Stabilizer (ECD 105)

Catalog No.	Bottle Size (mL)	Final Volume at 1x	# Plates at 100 $\mu$ L/well (9.6 mL/plate)	# Plates at 200 $\mu$ L/well (9.6 mL/plate)	Price (in US dollars)
ECD105-1	100	500 mL	50	25	\$220
ECD105-2	500	2.5 mL	255	126	\$695
ECD105-3	1,000	5 L	515	255	\$1,175
ECD105-4	10,000	50 L	5,200	2,600	\$10,900

#### ECD 200 Antigen-Down Conjugate Diluent and Stabilizer

5x Antigen-Down Conjugate Diluent and Stabilizer (ECD 205)

Catalog No.	Bottle Size (mL)	Final Volume at 1x	# Plates at 100 $\mu$ L/well (9.6 mL/plate)	# Plates at 200 $\mu$ L/well (9.6 mL/plate)	Price (in US dollars)
ECD205-1	100	500 mL	50	25	\$220
ECD205-2	500	2.5 L	255	126	\$695
ECD205-3	1,000	5 L	515	255	\$1,175
ECD205-4	10,000	50 L	5,200	2,600	\$10,900

**ECD 300 Polyclonal-Polyclonal Conjugate Diluent and Stabilizer**

5x Polyclonal – Polyclonal Conjugate Diluent and Stabilizer (x CD3)

Catalog No.	Bottle Size (mL)	Final Volume at 1x	# Plates at 100 $\mu$ L/well (9.6 mL/plate)	# Plates at 200 $\mu$ L/well (9.6 mL/plate)	Price (in US dollars)
ECD305-1	100	500 mL	50	25	\$490
ECD305-2	500	2.5 L	255	126	\$1,995
ECD305-3	1,000	5 L	515	255	\$3,700
ECD305-4	10,000	50 L	5,200	2,600	\$34,500

**ECD 400 Monoclonal – Goat Polyclonal Conjugate Diluent and Stabilizer**

5x Monoclonal – Goat Polyclonal HRP Conjugate Diluent and Stabilizer (ECD 405)

Catalog No.	Bottle Size (mL)	Final Volume at 1x	# Plates at 100 $\mu$ L/well (9.6 mL/plate)	# Plates at 200 $\mu$ L/well (9.6 mL/plate)	Price (in US dollars)
ECD405-1	100	500 mL	50	25	\$295
ECD405-2	500	2.5 L	255	126	\$895
ECD405-3	1,000	5 L	515	255	\$1,495
ECD405-4	10,000	50 L	5,200	2,600	\$12,995

**ECD 600 Conjugate Stock Stabilizing Reagent**

5x Conjugate Stock Stabilizing Reagent (ECD 605)

Catalog No.	Bottle Size (mL)	Final Volume at 1x	# Plates at 100 $\mu$ L/well (9.6 mL/plate)	#Plates at 200 $\mu$ L/well (9.6 mL/plate)	Price (in US dollars)
ECD605-1	100	500 mL	50	25	\$195
ECD605-2	500	2.5 L	255	126	\$595
ECD605-3	1,000	5 L	515	255	\$1,095
ECD605-4	10,000	50 L	5,200	2,600	\$8,895

## Sample Diluents

### Key Benefits

Sample Diluents improve assays by accurately reading and quantifying high-titer samples. High-titer samples may overload the finite binding capacity of the coated ELISA plate surface, thus requiring dilution prior to testing in the assay.

### Availability

The following Sample Diluents are available:

ESD 100 General-Purpose Sample Diluent - All ELISAs

Catalog No.	Bottle Size (mL)	# Samples Diluted 1:2 Using 250 $\mu$ L	# Samples Diluted 1:10 Using 450 $\mu$ L	Price (in US dollars)
ESD100-1	100	400	220	\$55
ESD100-2	500	2,000	1,100	\$165
ESD100-3	1,000	4,000	2,200	\$265
ESD100-4	10,000	40,000	22,200	\$2,100

For dilution of most serum, ascites, cell-culture supernatants, and antigens into the functional range of the ELISA

### ESD 200 Plasma Sample Diluent - Antigen-Down

For dilution of plasma samples into the functional range of the antigen-down ELISA to inhibit complement and thrombin (clotting) activity during the assay

Catalog No.	Bottle Size (mL)	# Samples Diluted 1:2 Using 250 $\mu$ L	# Samples Diluted 1:10 Using 450 $\mu$ L	Price (in US dollars)
ESD200-1	100	400	220	\$75
ESD200-2	500	2,000	1,100	\$230
ESD200-3	1,000	4,000	2,200	\$365
ESD200-4	10,000	40,000	22,200	\$2,900

### ESD 300 Pisces Sample Diluent

Catalog No.	Bottle Size (mL)	# Samples Diluted 1:2 Using 250 $\mu$ L	# Samples Diluted 1:10 Using 450 $\mu$ L	Price (in US dollars)
ESD300-1	100	400	220	\$75
ESD300-2	500	2,000	1,100	\$230
ESD300-3	1,000	4,000	2,200	\$365
ESD300-4	10,000	40,000	22,200	\$2,900

## Wash Buffers

### Key Benefits

Wash buffers improve assays as follows:

- Reduce non-specific background noise
- Increase the specific signal-to-noise ratio

### Availability

The following Wash Buffers are available:

Catalog No.	Bottle Size (mL)	Final Volume After Dilution	Price (in US dollars)
EWB100-1	100	1 L	\$40
EWB100-2	500	5 L	\$110
EWB100-3	1,000	10 L	\$240
EWB100-4	10,000	100 L	\$1,900

# Section 10

## Section 10: Contract Research Services

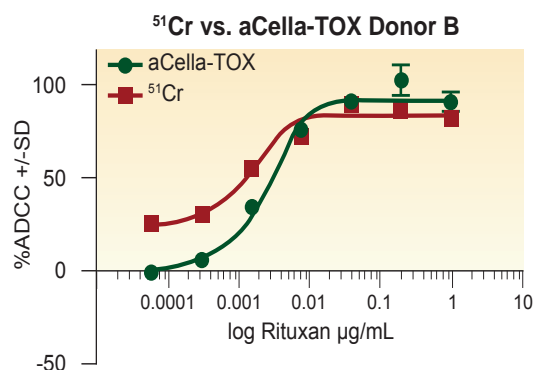
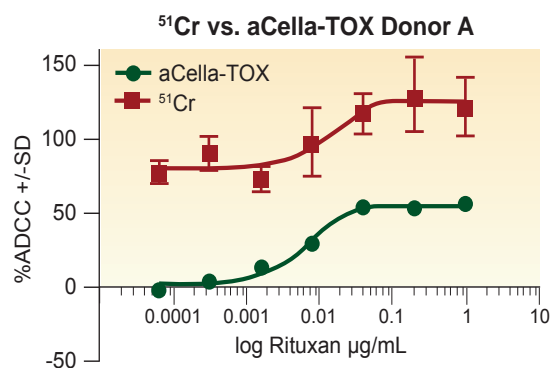
# ADCC / CDC Assay Services

## Cell Based Assay

### Antibody Dependent Cellular Cytotoxicity (ADCC) & Cell Complement Dependent Cytotoxicity (CDC)

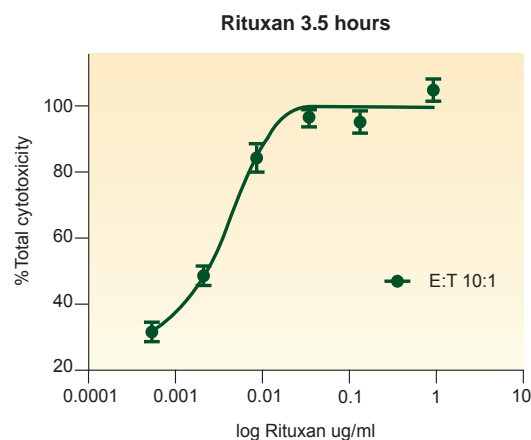
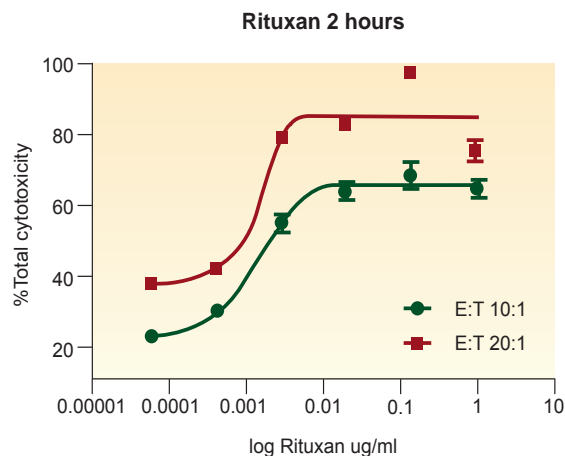
Cell Technology possesses a customizable ADCC/CMC assay platform that facilitates the selection of antibody drug candidates. Our bioluminescent ultra sensitive aCella-TOX Assay, is rapidly becoming the standard for ADCC/CMC assays within the therapeutics industry.

We provide ADCC assay services to determine the activity of the antibody against specified target cell lines. The result would be a dose dependent kinetic response of the therapeutic antibody. The assay service includes optimization of E/T ratios, measurement of ADCC activity at a series of antibody concentrations, at a certain E:T ratio. Correlation data with FACS analysis can be generated upon request.



**Donor A:** A Direct comparison of <sup>51</sup>Cr and aCella-TOX was carried out with the same donors with Daudi Cells. Log (EC50) value for aCella-Tox was -2.23, and that for <sup>51</sup>Cr was -2.087 for Donor A.

**Donor B:** A Direct comparison of <sup>51</sup>Cr and aCella-TOX was carried out with the same donors with Daudi Cells. Log (EC50) value for aCella-Tox was -2.612, and that for <sup>51</sup>Cr was -2.77 for Donor B.



**Figure:** 5000 Ramos cells/well were incubated with serially diluted Rituxan antibody for 15 minutes prior to the addition of purified NK cells stimulated overnight with IL-2.

The ADCC reaction was further incubated for 2 hours (Left graph) or 3.5 hours (Right graph) at the specified E:T ratios. % Cytotoxicity was measured using the aCella-

**Steps Involved for the ADCC implementation:**

1. Determination of optimal E:T ratios for the Target Cell line.
2. Procurement of the Antibody (or Antibodies) and Target Cell line (s) to be tested.
3. A dose response curve would be generated using aCella-TOX or FACS analysis or both. (as requested).

**Supplies and information to be provided by the client:**

1. Target Cell line(s) (adherent or suspension) (frozen vial(s)) and sufficient Antibody for the project.
2. Cell Culture Media and conditions for growth of the target cell line.
3. Antibody concentrations (if known).
4. Number of PBMC donors to be tested.
5. Information on Antigen expression Level on the target cell line (s) (FACS analysis can be done at the request of the client to determine this).

**Supplies to be provided by Cell Technology:**

All additional supplies would be procured by Cell Technology, and billed to the client at cost.

Final Report would be turned in after the Project with a dose response curve, showing Antibody dependence.

**Typical time frame is approximately 4 weeks / Cell line / Antibody**

For Schedule and Pricing, please call 888-727-7297 or 650-960-2170, or email us at [sales@technology.com](mailto:sales@technology.com).