CELL TECHNOLOGY

NOS^{TM}

Nitric Oxide Synthase (NOS) Detection Kit Protocol

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Precautions and Experimental Set up.

1. Prepare 1X-working solution of DAF-2DA immediately prior to use. Do not store any 1X solutions for later usage.
2. Keep DAF-2DA out of direct light.
3. After opening DAF-2DA reagent, aliquote and store at 4^0C.
4. **Keep the DAF-2DA reagent and stained samples protected from light. DAF-2DA is very light sensitive. When handling DAF-2DA or stained samples, it is advisable to work in a low light environment.**
5. MW: 446.4
6. Appearance: liquid (DMSO)
7. Prior to using DAF-2DA, spin down vial as contents may stick to the vial cap.

This reagent is considered hazardous and flammable.

Protocol

A. Cell Culture

1. If your samples are cultured in serum base media, you will need to wash your samples prior to adding the DAF-2DA dye. This wash step is necessary to remove serum/BSA components from the samples (see note below). You may wash your samples in Phosphate buffer or other buffers of your choice that do not contain serum and phenol red (e.g. Hanks Balanced Salt Solution).
Note: *Samples can be stained in black clear bottom 96 well plates for fluorescence plate reader or microscope application. Alternatively chamber slides maybe used for imaging of NOS activity.*
Note: *Serum, BSA and Phenol red may affect the fluorescence and loading of the dye into cells. This may interfere with the assay and thus should be used with caution.*

2. Make a 1x working solution of DAF-2DA by diluting it 1:250 to 1:500 in Phosphate buffer or other suitable buffers of your choice (e.g. Krebs Ringers Phosphate buffer, Hanks Balanced Salt Solution). The dilution buffer should have a pH of 7-7.5 with no serum/BSA and phenol red.
Note: *For optimal staining, each investigator should titrate out the reagent for their particular application. A minimum amount of dye concentration to stain your samples is desirable. This will give you adequate signal to noise separation.*
Note: *Serum, BSA and Phenol red may effect the fluorescence and interfere with the assay and thus should be used with caution.*

3. Next add the 1X DAF-2DA working solution to your experimental sample (from step 1) and incubate the samples for 30 to 60 minutes to pre load the dye. Reducing the incubation temperature will lessen the sub-cellular compartmentalization of the dye.
Note: *Each investigator should optimize the dye loading time.*

4. Wash your samples after step 3 to remove excess dye and re-suspend the samples in media or buffer of your choice.
Note: *Serum, BSA and Phenol red may effect the fluorescence thus interfere with the assay and thus should be used with caution.*

5. Samples are ready for experimental protocol. You may add NOS specific inhibitors prior to your experimental protocol.
Each individual researcher should determine optimal Experimental time.

6. NOS activity can be observed via a fluorescence microscope, fluorescence plate reader or flow cytometer. Excitation : 488nm and measure Emission at: 515 nm.

B. Tissue Sections

1. Tissue sections should be cut fresh or frozen sections made.
2. Do not fix sections with organic solvents or formalin as this will denature enzymes and give false readings from fixatives.
3. Sections can be placed in a slide chamber or other suitable staining vessel.
4. Wash the sections with serum/BSA and phenol free buffer (See above notes).
5. Make a 1X staining solution as in section A step 2.
6. Load the sections with the dye by flood the sections with the 1X dye solution and incubating for 1-2 hours.
7. After Wash the sections have been loaded with the dye, wash them to remove excess dye.
8. The sections are ready for experimental protocol.

Note: Keep The DAF-2DA reagent and stained sections protected from light at all times. DAF-2DA is very light sensitive.

References.

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