K – 5203

Immunofluorescent Assay Kit

for

inducible Nitric Oxide Synthase (iNOS or NOS Type 2)

R & D ®
Ab

For Research Use Only
Not for Use in Diagnostic Procedures

Research & Diagnostic Antibodies
Innovative Leaders in Immunoassay Technology

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1. Summary of Assay: Read all instructions before starting

This is an indirect immunofluorescent assay. The primary monoclonal was developed from mice immunized with human inducible nitric oxide (iNOS or NOS type 2). This monoclonal antibody has been shown to bind specifically to intact iNOS from humans, chimpanzees, monkeys, rats and mice. The secondary antibody was raised in goats, is specific for mouse IgG, and has been conjugated with FITC.

2. List of Components

Store the kit at 4°C until used. After the lyophilized powders are rehydrated, all the components should be stored at 4°C.

- IgG fraction of anti-iNOS clone 2A1-F8 ascites fluid: for 5.0 ml
- Antibody buffer: 11 ml
- Standard iNOS protein: for blocked controls
- Normal goat serum: for 20 ml
- Concentrated wash solutions #1 & #2: 2 x 20 ml
- Mounting medium with DABCO
- Affinity purified FITC-conjugated goat anti-mouse IgG serum: for 5.0 ml
- Instruction sheet

3. Preparation of Samples

A. Tissues: Perfuse tissue with the following three solutions: PBS, PBS containing 3% formaldehyde and 0.1% Triton X-100, and finally PBS. Freeze the tissue, prepare cryostat sections, and mount on glass slides. Wash the slides three times for 2 minutes in PBS which contains 0.1% Tritron X-100. Then follow the kit instructions contained on this sheet.
B. Cell Cultures: Wash the cells four times for 2 minutes in PBS pH 7.2, and then one fast rinse in distilled water. Drain well. Fix with 3% formaldehyde or neutral buffered formalin for 10 minutes, air dry and store frozen. Allow the slides to warm to room temperature before using.

4. Immunofluorescent Assay Procedure: Read Carefully

1. In a graduated cylinder dilute the 20 ml of concentrated Wash Solution #1 to 200 ml with distilled water to yield 0.9% NaCl with 0.1% Triton X-100. Dissolve the normal goat serum in 20 ml of the diluted wash solution #1, and then divide the remaining quantity of wash solution #1 into thirds by pouring 60 ml into each of three washing beakers or trays.
2. If the sample was fixed with an organic solvent, such as acetone, methanol or ethanol, no permeabilization is needed so proceed to step #3. However, if the sample was fixed with formaldehyde, formalin or glutaraldehyde, then it must be permeabilized before using this kit. To permeabilize the sample, soak it for 30 min in wash solution #1 prepared above, drain, and proceed to step #3.
3. Block the non-specific binding by applying 0.4 ml of the dilute normal goat serum to each sample (cover the entire sample). Let this stand for 15 min, drain, wash quickly in the first tray or beaker of wash solution #1, and drain.

4. Rehydrate the lyophilized anti-iNOS monoclonal antibody IgG which is the primary antibody for this assay with 5.0 ml of Antibody Buffer. Mix by inverting the bottle.
5. For blocked negative controls, dissolve the standard iNOS protein with 0.5 ml of the primary monoclonal antibody solution prepared in Step #4 above. Mix by inverting the bottle and pre-incubate for 30 minutes. Do **NOT** Add The Standard iNOS Protein To The Bottle Containing The Stock Anti-iNOS Monoclonal Antibody Since This Will Block All Antibody Binding In All Samples.
6. Apply 0.1 ml of the anti-iNOS monoclonal antibody to the fixed tissue or cells. Incubate 1 – 2 hours at room temperature.
7. For blocked negative controls, after pre-incubating the monoclonal antibody with the standard iNOS protein (see #5 above), apply 0.1 ml of the solution to the fixed tissue or cells. Incubate 1 – 2 hours at room temperature.
8. Wash the samples for 2 minutes in each of the three first washing solutions. Drain well after the final wash.
9. Rehydrate the FITC-conjugated second antibody with 5.0 ml of Antibody Buffer. Mix by inverting the bottle.
10. Apply 0.1 ml of the FITC-conjugated second antibody to each of the tissue or cell samples. Incubate 45 minutes at room temperature.
11. In a graduated cylinder dilute the 20 ml of concentrated Wash Solution #2 to 200 ml with distilled water to yield 0.9% NaCl with 0.1% Triton X-100. Pour 65 ml into each of three washing beakers or trays, and fill a fourth beaker with distilled water.
12. Wash the samples for 2 minutes in each of the three second washing solutions. Quickly rinse once in distilled water and drain well.
13. Mount a cover slip using the mounting medium (Ref. 1) which contains DABCO (Ref. 2) to stop fading.
14. Observe the fluorescent staining using a fluorescent microscope with excitation and emission wavelengths set for FITC.

5. Specificity of the Assay.

Protein	% Cross Reactivity
hiNOS (NOS type 2)	100
rhnNOS (NOS type 1)	0
rheNOS (NOS type 3)	0
Cytochrome P450	0
ACTH(1-39)	0
Calcitonin	0
PTH (1-84)	0

6. References

1. Heimer and Taylor (1974) J Clin Path, 27: 254
2. Johnson, et al (1982) J Immunol Meth, 55: 231

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