

K – 1688

Immunofluorescent Assay Kit

for

heNOS(NOS Type 3)

R & D®
Ab

**For Research Use Only
Not for Use in Diagnostic Procedures**

Research & Diagnostic Antibodies

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1. Summary of Assay: Read all instructions before starting

This is an indirect immunofluorescent assay. The primary antibody was raised in rabbits immunized with a synthetic peptide analogue of heNOS(2-11): this primary antibody has been shown to bind specifically to heNOS(2-11). The secondary antibody was raised in goats, is specific for rabbit 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 rabbit heNOS(2-11) anti-serum: for 5.0 ml
- Affinity purified FITC-conjugated goat anti-rabbit IgG serum: for 5.0 ml
- heNOS(2-11) specific synthetic peptide antigen: for 0.50 ml
- Normal goat serum: for 20 ml
- Antibody buffer: 11 ml
- Concentrated wash solutions #1 & #2: 2 x 20 ml
- Mounting medium with DABCO
- 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. (Note: Fixing the tissue with ice cold acetone after mounting on glass slides may improve the performance of this kit.) Wash the slides three times for 2 minutes in PBS which contains 0.1% Triton 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 ice cold acetone or neutral buffered formalin for 10 minutes, dry and store frozen. Allow the slides to warm to room temperature before using, then follow the kit instructions contained on this sheet.

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 rabbit anti-heNOS(2-11) 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 heNOS(2-11) specific synthetic peptide with 0.5 ml of the primary antibody solution, rabbit anti-heNOS(2-11) IgG solution prepared in Step #4 above. Mix by inverting the bottle and pre-incubate for 30 minutes. Do **NOT** Add The heNOS(2-11) Specific Peptide to the To The Bottle Containing The Stock Primary Antibody Since This Will Block All Antibody Binding In All Samples.
6. Apply 0.1 ml of the primary antibody to the fixed tissue or cells. Incubate 1 – 2 hours at room temperature.
7. For blocked negative controls, after pre-incubating the antibody with the heNOS(2-11) specific peptide (see #5 above), apply 0.1 ml of the solution to the fixed tissue or cells. Incubate 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

The antiserum was raised in a rabbit that was immunized with heNOS(2-11) covalently attached onto a carrier protein, and it has been characterized by immunocytochemical, ELISA, and western blot techniques. The antiserum has been found to be highly specific for this peptide sequence and is suitable for the immunocytochemical detection of heNOS(2-11).

Polypeptide	% Cross Reactivity
heNOS(2-11)	100

6. References

1. Heimer and Taylor (1974) J Clin Path, 27: 254
2. Johnson, et al (1982) J Immunol Meth, 55: 231 or see the Frequently Ask Questions (FAQ) page on our web site at www.RDAbs.com/faq.htm

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(last revision 2/02)