K – 5646

Immunofluorescent Assay Kit

for

Beta 2 Nicotinic Receptor

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 antibody was raised in rabbits immunized with a synthetic peptide analogue of the carboxyl terminal of the β2 nicotinic receptor conjugated onto a carrier protein: this primary antibody has been shown to bind specifically to the β2 nicotinic receptor. 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 anti-β2 nicotinic receptor antiserum: for 5.0 ml
- Affinity purified FITC-conjugated goat anti-rabbit IgG serum: for 5.0 ml
- β2 nicotinic receptor 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, PLP (Periodate/Lysine/Paraformaldehyde - see ref. 3 or the Frequently Asked Questions (FAQ) page on our web site at www.RDAbs.com/faq.htm), and finally PBS. Post-fix for 2 hours in PLP, then 1 hour in PBS, and finally soak in 30% sucrose overnight or until the tissue sinks. 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% 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 for 10 minutes with PLP (Periodate/Lysine/Paraformaldehyde - see ref. 3 or the Frequently Asked Questions (FAQ) page on our web site at www.RDAbs.com/faq.htm), 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. 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.

3. Rehydrate the lyophilized rabbit anti-β2 nicotinic receptor IgG which is the primary antibody for this assay with 5.0 ml of Antibody Buffer. Mix by inverting the bottle.
4. For blocked negative controls, dissolve the β2 nicotinic receptor specific synthetic peptide with 0.5 ml of the primary antibody solution, rabbit anti-β2 nicotinic receptor IgG solution prepared in Step #4 above. Mix by inverting the bottle and pre-incubate for 30 minutes. Do **NOT** Add The β2 Nicotinic Receptor Specific Peptide to the To The Bottle Containing The Stock Primary Antibody Since This Will Block All Antibody Binding In All Samples.
5. Apply 0.1 ml of the primary antibody to the fixed tissue or cells. Incubate 1 – 2 hours at room temperature.
6. For blocked negative controls, after pre-incubating the antibody with the β2 nicotinic receptor specific peptide (see #5 above), apply 0.1 ml of the solution to the fixed tissue or cells. Incubate 2 hours at room temperature.
7. Wash the samples for 2 minutes in each of the three first washing solutions. Drain well after the final wash.
8. Rehydrate the FITC-conjugated second antibody with 5.0 ml of Antibody Buffer. Mix by inverting the bottle.
9. Apply 0.1 ml of the FITC-conjugated second antibody to each of the tissue or cell samples. Incubate 45 minutes at room temperature.
10. 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.
11. Wash the samples for 2 minutes in each of the three second washing solutions. Quickly rinse once in distilled water and drain well.
12. Mount a cover slip using the mounting medium (Ref. 1) which contains DABCO (Ref. 2) to stop fading.
13. 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 with was immunized with a peptide analogue of the carboxyl terminus of β2 nicotinic receptor 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 the β2 nicotinic receptor.

Polypeptide	% Cross Reactivity
β2 Nicotinic Receptor (493-502))	100
β2 Nicotinic Receptor	~90
Other Nicotinic Receptor Subtypes	0

6. References

1. Heimer and Taylor (1974) J Clin Path, 27: 254
2. Johnson, et al (1982) J Immunol Meth, 55: 231
3. McLean and Nakane (1974) J Histochem Cytochem, 22: 1077 or see the

Frequently Ask Questions (FAQ) page on our web site at www.RDAbs.com/faq.htm

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