R&D ® Ab

Research & Diagnostic Antibodies

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IgG Fraction Rabbit Anti-Muscarinic M_4 Receptor Serum

AS-3761G Lot # 9144

This IgG fraction was prepared by precipitation, dialysis and chromatography from an antiserum that was raised in a rabbit which was immunized with a peptide analogue of the carboxyl terminal of the M4 receptor covalently attached onto a carrier protein. The antiserum has been shown to be specific for the COOH terminal of the M_4 receptor and has been characterized by western blotting, ELISA, and cell staining techniques. This IgG fraction is suitable for immunocytochemical and western immunoblotting detection of the receptor. Dilute the lyophilized antibody with 0.1 ml of 10 mg/ml BSA in PBS for the equivalent concentration in whole antiserum, or with additional buffer for more dilute antibody. This vial contains 3.0 mg of purified IgG.

Antiserum Specificity

Polypeptide	% Cross Reactivity
M_4 Muscarinic Receptor (469-478)	100
M_4 Muscarinic Receptor	70
M_1 Muscarinic Receptor(451-460)	0
M_1 Muscarinic Receptor	0
M_2 Muscarinic Receptor(457-466)	0
M_2 Muscarinic Receptor	0
M_3 Muscarinic Receptor(580-589)	0
M_3 Muscarinic Receptor	0
M_5 Muscarinic Receptor(519-531)	0
M_5 Muscarinic Receptor	0

Immunocytochemical Staining

The purified IgG has been found to stain PLP fixed cultured cells known to express the muscarinic M_4 receptor at a concentration of 50 μg/ml. For information on PLP fixative see the FAQ page on our Web site at www.RDAbs.com

Western Immunoblot

Western immunoblots using whole rat brain homogenate and cultured cells known to express the M4 have been successful at a concentration of 80 μg/ml.

Western Blotting Protocol

1. After SDS-PAGE (on either 4-15% gradient gels or single percentage gels, such at 12% gels) and electrophoretic transfer to PVDF membrane, block the membrane overnight with 2% normal goat serum in TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. Apply the IgG fraction after diluting to at least 80 μg/ml (Note: higher dilutions may be needed). Use 2% normal goat serum in TBS/Tween-20 as buffer for the primary antibody. Let the primary antibody bind for 2-4 hours. Wash x 3 with TBS/Tween-20.
4. Apply affinity purified HRP-goat anti-rabbit IgG antiserum diluted 1:2500 (dilution may vary depending upon supplier) in 2% normal goat serum in TBS/Tween-20. Incubate 1-2 hours. Note: greater sensitivity may be achieved using ABC techniques.
5. Wash x 4 for 5 minute/wash with TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.