R&D ® Ab
Research & Diagnostic Antibodies
2645 W. Cheyenne Ave, N. Las Vegas, NV 89032 Phone: 702-638-7800

IgG Fration of Rabbit Anti-Muscarinic M_3 Receptor Serum

AS-3741G Lot # 10530

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 M_3 receptor covalently attached onto a carrier protein. The antiserum has been shown to be specific for the COOH terminal of the M_3 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 2.78 mg of purified IgG.

Antiserum Specificity

Polypeptide	% Cross Reactivity
M3 Muscarinic Receptor (580-589)	100
M3 Muscarinic Receptor	70
M1 Muscarinic Receptor(451-460)	0
M1 Muscarinic Receptor	0
M2 Muscarinic Receptor(457-466)	0
M2 Muscarinic Receptor	0
M4 Muscarinic Receptor(469-478)	0
M4 Muscarinic Receptor	0
M5 Muscarinic Receptor(519-531)	0
M5 Muscarinic Receptor	

Immunocytochemical Staining

This IgG fraction at a concentration of 10 μg/ml has been found to stain rat brain sections that are known to express the muscarinic M_3 receptor after fixing with PLP fixative and using ABC techniques. Note: a reference for the PLP fixative can be found on the frequently asked questions (FAQ) page of our web site at www.RDAbs.com.

Western Immunoblot

Western immunoblots using whole rat brain homogenate have been successful at a concentration of 50 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 overnight with 4% normal goat serum using TBS/Tween-20 buffer as diluent.
2. Wash x 2 with TBS/Tween-20.
3. Apply the rabbit polyclonal antibody after dilution to at least 50 μg/ml (Note: higher dilutions may be needed). Use 2% normal goat serum in TBS/Tween-20. Let the primary antibody bind for 2-4 hours.
4. Wash x 3 with TBS/Tween-20.
5. 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.
6. Wash x 4 for 5 minutes per wash cycle with TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.