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

IgG Fraction of Rabbit Anti-M_1 Receptor Serum

AS-3701G Lot # 8360

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_1 receptor covalently attached onto a carrier protein. The antiserum has been shown to be specific for the COOH terminal of the M_1 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 1.58 mg of purified IgG.

Antiserum Specificity

Polypeptide	% Cross Reactivity
M1 Muscarinic Receptor (451-460)	100
M1 Muscarinic Receptor	60
M2 Muscarinic Receptor (457-466)	0
M2 Muscarinic Receptor	0
M3 Muscarinic Receptor (580-589)	0
M3 Muscarinic Receptor	0
M4 Muscarinic Receptor (469-478)	0
M4 Muscarinic Receptor	0
M5 Muscarinic Receptor (519-531)	0
M5 Muscarinic Receptor	0

Immunocytochemical Staining

This IgG fraction has been found to stain PLP fixed rat brain sections known to express the muscarinic M_1 receptor using ABC techniques at a concentration of 5 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 have been successful at a concentration of 20 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, block the 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 IgG fraction after diluting to at least 20 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.
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 minute per wash cycle in TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.