

IgG Fraction Rabbit Anti-Muscarinic M₅ Receptor Serum

AS-3781G

Lot # 9145

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 M₅ receptor covalently attached onto a carrier protein. The antiserum has been shown to be specific for the COOH terminal of the M₅ 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 2.5 mg of purified IgG.

Antiserum Specificity

Polypeptide	% Cross Reactivity
M ₅ Muscarinic Receptor (519-531)	100
M ₅ Muscarinic Receptor	75
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 ₄ Muscarinic Receptor(469-478)	0
M ₄ Muscarinic Receptor	0

Immunocytochemical Staining

The purified IgG has been found to stain PLP fixed cultured cells known to express the muscarinic M₅ receptor at a concentration of 100 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 M₅ receptor have been successful using this pooled antisera 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 as 12% gels) and electrophoretic transfer to PVDF membrane, 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 50 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 1% 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 in TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.