

Rabbit Anti-Muscarinic M₁ Receptor Serum

AS-3701S

Lot # 10411

The antiserum was raised in a rabbit which was immunized with a peptide analogue of the carboxyl terminal of the muscarinic M₁ receptor covalently attached onto a carrier protein. This antiserum is specific for the COOH terminal of the M₁ receptor and is suitable for immunocytochemical and western immunoblotting detection of the receptor. The antiserum has been characterized by western blotting, ELISA, and cell staining techniques. Dilute the lyophilized antiserum with 0.1 ml of 10 mg/ml BSA in PBS for the equivalent of whole antiserum, or with additional buffer for more dilute antiserum.

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 antiserum has been found to stain PLP fixed rat brain sections known to express the muscarinic M₁ receptor using ABC techniques at a dilution of 1:5000. 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 dilution of 1:800.

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 2% normal goat serum in TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. Apply the rabbit polyclonal antibody after dilution to at least 1:800 (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/wash with TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.