R&D® Ab

Research & Diagnostic Antibodies

2645 W Cheyenne Ave., North Las Vegas, NV 89032 Phone: 702-638-7800

Rabbit Anti-Muscarinic M_3 Receptor Serum

AS-3741S Lot # 10573

The antiserum was raised in a rabbit which was immunized with a peptide analogue of the carboxyl terminal of the muscarinic M_3 receptor covalently attached onto a carrier protein. This antiserum is specific for the COOH terminal of the M_3 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 is PBS for the equivalent of whole antiserum, or with additional buffer for more dilute antiserum.

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	0

Immunocytochemical Staining

This antiserum has been found to stain PLP fixed rat brain sections known to express the muscarinic M_3 receptor using ABC techniques at a dilution of 1:2500. For information on PLP fixative see the FAQ page on our web site at RDAbs.com

Western Immunoblot

Western immunoblots using whole rat brain homogenate have been successful at a dilution of 1:500.

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 rabbit polyclonal antibody after dilution to at least 1:500 (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.