R&D Ab
Research & Diagnostic Antibodies
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Rabbit Anti-Muscarinic M_5 Receptor Serum

AS-3781S Lot # 9064

The pooled antisera were raised in rabbits which were immunized with a peptide analogue of the carboxyl terminal of the muscarinic M_5 receptor covalently attached onto a carrier protein. This antibody is specific for the COOH terminal of the M_5 receptor and is suitable for immunocytochemical and western immunoblotting detection of this receptor. The antibody has been characterized by western blotting, ELISA, and cell staining techniques. Dilute the lyophilized pooled antisera with 0.1 ml of 10 mg/ml BSA is PBS for the equivalent of whole antisera, or with additional buffer for more dilute antisera.

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 pooled antisera has been found to stain PLP fixed rat brain sections known to express the muscarinic M_5 receptor at a dilution of 1:400. 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 M_5 receptor have been successful using this pooled antisera at a dilution of 1:400.

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 1:400 (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.