

**Rabbit Anti-Muscarinic M<sub>2</sub> Receptor Serum**

AS-3721S

Lot # 8216

The antiserum was raised in a rabbit which was immunized with a peptide analogue of the carboxyl terminal of the muscarinic M<sub>2</sub> receptor covalently attached onto a carrier protein. This antiserum is specific for the COOH terminal of the M<sub>2</sub> 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**

<b>Polypeptide</b>	<b>% Cross Reactivity</b>
M2 Muscarinic Receptor (457-466)	100
M2 Muscarinic Receptor	50
M1 Muscarinic Receptor(451-460)	0
M1 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 M<sub>2</sub> 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](http://www.RDAbs.com)

**Western Immunoblot**

Western immunoblots using whole rat brain homogenate have been successful at a dilution of 1:1000.

**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 1:1000 (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.