

Western Immunoblotting Reagents: IgG Fraction of Rabbit Anti-M₃ Receptor Serum

WR-3741

Lot # 8483

The antiserum was raised in a rabbit which was immunized with a peptide analogue of the carboxyl terminal of the M₃ receptor covalently attached onto a carrier protein. The IgG fraction of the rabbit antiserum was prepared by precipitation, dialysis, and column chromatography. Rehydrate the lyophilized IgG fraction with 5.0 ml of 10 mgm/ml BSA in PBS. The stock solution should be further diluted 1:8 with additional buffer prior to use (see below). This should be sufficient for at least 20 lanes. This antiserum has been found to stain specifically the M₃ receptor in western immunoblots of whole rat brain homogenates. The antiserum was tested for recognition of the other muscarinic receptor subtypes by ELISA and immunocytochemical techniques.

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

Western Blotting Protocol

1. After SDS-PAGE (on either 4-15% gradient gels or single percentage gels, such as 7.5% 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. For blocked antibody controls dissolve 150 nmoles of peptide PS-3741B in 600 µl of reconstituted antibody. Incubate one hour. Then add 5.4 ml of 2% normal goat serum in TBS/Tween-20. Using 2 ml per lane this should be sufficient for 3 blocked control lanes. **DO NOT ADD THE PEPTIDE TO THE STOCK POLYCLONAL ANTIBODY. THIS WILL BLOCK ALL BINDING.**
4. Apply the rabbit IgG after dilution to at least 1:8 (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 1 - 2 hours.
5. Wash x 3 with TBS/Tween-20.
6. 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.
7. Wash x 4 for 5 min/wash with TBS/Tween-20.
8. Develop color using the enhanced DAB reaction.

PS-3741B: Muscarinic M₃ Receptor (Cys⁵⁷⁹-580-589)

Amino Acid Sequence:

Cys-Phe-His-Lys-Arg-Val-Pro-Glu-Gln-Ala-Leu

Mol. Wt.: 1327.6

Peptide Purity: > 95%

Peptide Quantity: 150 nmoles

Peptide Lot # 9520

HPLC Analysis: See Attached Chart Recording

Solvent System: A. 0.05 M KH₂PO₄, pH 3.0

B. 70% Acetonitrile + 30% A

Solvent Program:

<u>Time</u>	<u>Flow</u>	<u>%A</u>	<u>%B</u>
0	1.2	100	0
30	1.2	25	75
31	1.2	0	100
32	1.2	100	0
35	1.2	100	0

Detection: optical density at 225 nm

Results: single peak at R_t = 13.005 min