

**Western Immunoblotting Reagents:
IgG Fraction of Rabbit Anti-M₂ Receptor Serum**

WR-3721

Lot # 9180

The antiserum was raised in a rabbit which was immunized with a peptide analogue of the carboxyl terminal of the M₂ 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 M₂ 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
M ₂ Receptor (457-466)	100
M ₂ Receptor	80
M ₁ Receptor	0
M ₃ Receptor	0
M ₄ Receptor	0
M ₅ 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-3721 in 600 µl of reconstituted antibody. Incubate one hour. Then add 5.4 ml of 2% normal goat serum in TBS-Tween. Using 2 ml per lane this should be sufficient for 3 blocked control lanes. **DO NOT ADD THE PEPTIDE TO THE STOCK POLYCLONAL ANTIBODY SINCE 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/cycle with TBS/Tween-20.
8. Develop color using the enhanced DAB reaction.

PS-3721: Muscarinic Receptor M₂ (457-466)

Amino Acid Sequence:

Cys-His-Tyr-Lys-Asn-Ile-Gly-Ala-Thr-Arg

Mol. Wt.: 1162.4

Peptide Purity: 95%

Peptide Quantity: 150 nmoles

Peptide Lot # 1994

HPLC Analysis: See Attached Chart Recording

Solvent System: A. 0.05 M KH₂PO₄, pH 3.0
B. 70% AcCn + 30% A

Solvent Program:	<u>Time</u>	<u>Flow</u>	<u>%A</u>	<u>%B</u>
	0	1.2	100	0
	40		60	40
	42		0	100
	45		0	100
	46		100	0
	50		100	0

Detection: optical density at 225 nm

Results: single peak at R_t = 10.022 min