

**Western Immunoblotting Reagents:  
IgG Fraction of Rabbit Anti-M<sub>5</sub> Receptor Serum**

WR-3781

Lot # 9184

The antiserum was raised in a rabbit which was immunized with a peptide analogue of the carboxyl terminal of the M<sub>5</sub> 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 M<sub>5</sub> 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**

<b>Polypeptide</b>	<b>% Cross Reactivity</b>
M <sub>5</sub> Receptor (516-531)	100
M <sub>5</sub> Receptor	80
M <sub>1</sub> Receptor	0
M <sub>2</sub> Receptor	0
M <sub>3</sub> Receptor	0
M <sub>4</sub> 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 4% normal goat serum in TBS/Tween-20 buffer as diluent.
2. Wash x 2 with TBS/Tween-20.
3. For blocked antibody controls dissolve 150 nmoles of peptide PS-3783 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. 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 in TBS/Tween-20.
8. Develop color using the enhanced DAB reaction.

**PS-3783: Muscarinic Receptor M<sub>5</sub> (516-531)**

Amino Acid Sequence:

NH<sub>2</sub>-Lys-Lys-Val-Glu-Glu-Lys-Leu-Tyr-Trp-Gln-Gly-Asn-Ser-Lys-Leu-Pro-COOH

Mol. Wt.: 1947.3

Peptide Purity: 95%

Peptide Quantity: 150 nmoles

Peptide Lot # 8251

HPLC Analysis: See Attached Chart Recording

Solvent System: A. 0.05 M KH<sub>2</sub>PO<sub>4</sub>, 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
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<sub>t</sub> = 15.858 min