R&D® Ab

Research & Diagnostic Antibodies

2645 W. Cheyenne Ave, N. Las Vegas, NV 89032 Phone: 702-638-7800

Western Immunoblotting Reagents: IgG Fraction of Rabbit Anti-M_4 Receptor Serum

WR-3761 Lot # 9182

The antiserum was raised in a rabbit which was immunized with a peptide analogue of the carboxyl terminal of the M_4 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_4 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_4 Receptor (460-478)	100
M_4 Receptor	80
M_1 Receptor	0
M_2 Receptor	0
M_3 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 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-3763 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 per cycle in TBS/Tween-20.
8. Develop color using the enhanced DAB reaction.

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PS-3763: Muscarinic Receptor M_4 (460-478)

Amino Acid Sequence:

NH_2-Lys-Lys-Thr-Phe-Arg-His-Leu-Leu-Leu-Ser-Gln-Tyr-Arg-Asn-Ile-Gly-Thr-Ala-Arg-COOH

Mol. Wt.: 2302.7
Peptide Purity: 98%
Peptide Quantity: 150 nmoles
Peptide Lot# 8250

HPLC Analysis: See Attached Chart Recording
Solvent System: A. 0.05 M KH_2PO_4, pH 3.0
B. 70% AcCn + 30% A

Solvent Program:

Time	Flow	%A	%B
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 = 18.020 min