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-Mu Opioid Receptor Serum

WR-3942 Lot # 8292

The antiserum was raised in a rabbit which was immunized with a peptide analogue of the carboxyl terminal of mu opioid 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 TBS/Tween 20 that contains 1% normal goat serum(NGS). 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 mu opioid receptor in western immunoblots of whole rat brain homogenates. The antiserum was tested for recognition of the other opioid receptor subtypes by ELISA and immunocytochemical techniques.

Antiserum Specificity

Polypeptide	% Cross Reactivity
Mu Opioid Receptor (391-398)	100
Mu Opioid Receptor	~50
Delta Opioid Receptor	0
Kappa Opioid Receptor	0

Western Blotting Protocol

1. After SDS-PAGE (on either 4-15% gradient gels or single percentage gels, such as 10% gels) and electrophoretic transfer to PVDF membrane, block the membrane overnight with 4% normal goat serum using TBS/Tween-20 buffer as diluent.
2. Wash x 2 with TBS/Tween-20.
3. For blocked antibody controls dissolve 150 nmole of peptide PS-3942 in 600 µ l of reconstituted stock antibody. Incubate one hour. Then add 5.4 ml of 2% normal goat serum in TBS-Tween and use 2.0 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 polyclonal antibody after dilution to at least 1:8 (Note:higher dilutions may be needed). Use 2% normal goat serum in TBS/Tween 20 with 2% NGS as buffer for the primary antibody. Let the primary antibody bind for 2-4 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 minutes per wash cycle of TBS/Tween-20.

Develop color using the enhanced DAB reaction.Quality Control Data Sheet

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PS-3942B-2: Mu Opioid Receptor (Cys^{390}-391-398)

Amino Acid Sequence:

NH_2-Cys-Glu-Ala-Glu-Thr-Ala-Pro-Leu-Pro-COOH

Mol. Wt.: 930.1
Peptide Quantity: 150 moles
Peptide Purity: > 95% by HPLC
Lot Number: 10475

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	1.0	100	0
40	1.0	60	40
42	1.0	0	100
45	1.0	100	0
46	1.0	100	0

Detection: optical density at 225 nm
Results: Major peak at R_t = 20.030 min