

## Western Immunoblotting Reagents: IgG Fraction of Rabbit Anti-Delta Opioid Receptor Serum

WR-3922

Lot # 8288

The antiserum was raised in a rabbit which was immunized with a peptide analogue of the carboxyl terminal of the delta 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's with 5.0 ml of TBS/Tween-20 buffer that contains 1% normal goat serum (NGS). This 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 antibody has been found to stain specifically the delta 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
Delta Opioid Receptor (360-372)	100%
Delta Opioid Receptor	~60%
Mu 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-3922 in 600 µl of reconstituted stock antibody. Incubate one hour. Then add 5.4 ml of 1% 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 polyclonal antibody after dilution to at least 1:8 (Note: higher dilutions may be needed). Use 1% normal goat serum in TBS/Tween-20 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 1% normal goat serum TBS/Tween-20. Incubate 1-2 hours.
7. Wash x 4 for 5 min per wash cycle in TBS/Tween-20.
8. Develop color using the enhanced DAB reaction.

**PS-3922: Delta Opioid Receptor (360-372)**

Amino Acid Sequence:

Cys-Thr-Pro-Ser-Asp-Gly-Pro-Gly-Gly-Gly-Ala-Ala-Ala

Mol. Wt.: 1060.1

Peptide Purity: > 92% (see HPLC chart recording)

Peptide Quantity: 150 nmoles

Peptide Lot #4903

HPLC Analysis: See 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> = 12.047 min