

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

WR-3964

Lot # 8364

The antiserum was raised in a rabbit which was immunized with a peptide analogue of the carboxyl terminal of the kappa 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 2% 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 kappa 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
Kappa Opioid Receptor (346-380)	100
Kappa Opioid Receptor	~90
Delta Opioid Receptor	0
Mu 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-3965 in 600 µl of reconstituted stock antibody. Incubate one hour. Then add 5.4 ml of 2% normal goat serum in TBS/Tween-20 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 diluting the stock solution 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 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.
8. Develop color using the enhanced DAB reaction.

## PS-3965: Rat Kappa Opioid Receptor (Tyr<sup>345</sup>-346-380)

Amino Acid Sequence:

NH<sub>2</sub>-Tyr-Phe-Pro-Ile-Lys-Met-Arg-Met-Glu-Arg-Gln-Ser-Thr-Asn-Arg-Val-Arg-Asn-  
Thr-Val-Gln-Asp-Pro-Ala-Ser-Met-Arg-Asp-Val-Gly-Gly-Met-Asn-Lys-Pro-Val-COOH

Mol. Wt.: 4210.9

Peptide Purity: > 95% (see HPLC chart recording)

Peptide Quantity: 150 nmoles

Peptide Lot # 7963

HPLC Analysis:

Solvent System: A. 50 mM KH<sub>2</sub>PO<sub>4</sub> pH 3.0  
B. 70% Acetonitrile + 30% A

Solvent Program:	Time	Flow	%A	%B
	0	1.2	100	0
	24		40	60
	25		0	100
	26		100	0
	29		100	0

Detection: optical density at 225nm

Results: single peak at R<sub>t</sub> = 18.120 min