

Western Immunoblotting Reagents:
IgG Fraction of Rabbit Anti-PKC β 1 Serum
WR-2423 Lot # 6535

The antiserum was raised in a rabbit which was immunized with a peptide analogue of Protein Kinase C β 1(661-671) 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 PKC β 1 in western immunoblots of whole rat brain homogenate. The antiserum was tested for recognition of the other Protein Kinase C isozymes by immunocytochemical and ELISA techniques.

Antiserum Specificity

Polypeptide	% Cross Reactivity
Protein Kinase C β 1	100
Protein Kinase C α	0
Protein Kinase C β 2	< 0.2
Protein Kinase C δ	0
Protein Kinase C γ	0
Protein Kinase C ϵ	0
Protein Kinase C ζ	0
Protein Kinase C η	0
Protein Kinase C θ	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-2421 in 600 μ l of reconstituted stock antibody. Incubate one hour. Then add 5.4 ml of 1% 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 dilution to at least 1:8 (Note: higher dilutions may be needed). Use 1% normal goat serum in TBS/Tween-20 with 1% 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 1% 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-2421: Protein Kinase C β 1 [661-671] Rat
Lot# 2072**

Amino Acid Sequence: Ser-Tyr-Thr-Asn-Pro-Glu-Phe-Val-Ile-Asn-Val

Mol. Wt.: 1282.3

Peptide Purity: > 98% (by HPLC analysis)

Peptide Quantity: 150 nmoles

Date: Feb. 22, 1991

HPLC Analysis

Solvent System: A. 0.1% TFA in water

B. 0.1% TFA in acetonitrile

Solvent Program:	<u>Time</u>	<u>Flow</u>	<u>%A</u>	<u>%B</u>
	0.0	1.2	100	0
	2.0		100	0
	17.0		0	100
	18.0		0	100
	20.0		100	0
	22.0		100	0

Detection: optical density at 271 nm

Results: single peak at $R_t = 12.85$ min