

# **Research & Diagnostic Antibodies**

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# $We stern\ Immunoblotting\ Reagents:$ $IgG\ Fraction\ of\ Rabbit\ Anti-PKC\ \beta 2[660\text{-}672]\ Serum$

WR-2428 Lot # 6531

The antiserum was raised in a rabbit which was immunized with a peptide analogue of Protein Kinase C  $\beta2$  (660-672) 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  $\beta2$  in western immunoblots of whole rat brain homogenates. 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 β2	100	
Protein Kinase C α	0	
Protein Kinase C β1	0	
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 in TBS/Tween-20 buffer.
- 2. Wash x 2 with TBS/Tween-20.
- 3. For blocked antibody controls dissolve 150 nmole of peptide PS-2426 in 600 μl 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-2426: Protein Kinase C β2 [660-673] (Rat) Lot# 2068

Amino Acid Sequence: Ser-Phe-Val-Asn-Ser-Glu-Phe-Leu-Lys-Pro-Glu-Val-

Lys-Ser

Mol. Wt.: 1610.9

Peptide Purity: > 98% (by HPLC analysis)

Peptide Quantity: 150 nmole

Date: Feb. 22, 1991

## **HPLC Analysis**

Solvent System: A. 0.1% TFA in water

B. 0.1% TFA in acetonitrile

Solvent Program: Time Flow %A %B

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 225 nm Results: single peak at  $R_t = 12.29$