

**Western Immunoblotting Reagents:  
IgG Fraction of Rabbit Anti-PKC-Zeta Serum**

WR-2463

Lot # 6756

The antiserum was raised in a rabbit which was immunized with a peptide analogue of Protein Kinase C Zeta (480-492) 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-Zeta in western immunoblots of whole rat brain homogenates. The antiserum was tested for recognition of the other Protein Kinase C isozymes by ELISA and immunocytochemical techniques.

**Antiserum Specificity**

<b>Polypeptide</b>	<b>% Cross Reactivity</b>
Protein Kinase C Zeta	100
Protein Kinase C Alpha	0
Protein Kinase C Beta 1	0
Protein Kinase C Beta 2	0
Protein Kinase C Gamma	0
Protein Kinase C Delta	0
Protein Kinase C Epsilon	0
Protein Kinase C Eta	0
Protein Kinase C Thete	0

**Western Blotting Protocol**

1. After SDS-PAGE (on either 4-15% gradient gels or single percentage gels, such as 12% gels) and electrophoretic transfer to PVDF membrane, block the membrane overnight with 2% 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-2461 in 600 µl of reconstituted stock antibody. Incubate 45 - 60 min. Then add 5.4 ml of 2% NGS 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 ANTIBODY SINCE THIS WILL BLOCK ALL BINDING.**
4. Apply the rabbit antibody after diluting the stock 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. Note: greater sensitivity may be achieved using ABC techniques.
7. Wash x 4 for 5 minute/wash with TBS/Tween-20.
8. Develop color using the enhanced DAB reaction.

**PS-2461: Protein Kinase C zeta [Tyr<sup>479</sup>-480-492]**

Amino Acid Sequence:

NH<sub>2</sub>-Tyr-Ile-Asn-Pro-Leu-Leu-LeuSer-Ala-Glu-Glu-Ser-Val-COOH

Mol. Wt.: 1447.7

Peptide Purity: > 98% (see attached HPLC analysis)

Peptide Quantity: 150 nmoles

Lot # 2050

Date: Feb. 5, 1991

HPLC analysis

Solvent System: A. 0.05 M KH<sub>2</sub>PO<sub>4</sub>, pH 3.0  
B. 70% Acetonitrile + 30% A

Solvent Program:	<u>Time</u>	<u>Flow</u>	<u>% A</u>	<u>% B</u>
	0	1.2	100	0
	2		100	0
	17		0	100
	18		0	100
	20		100	0
	22		100	0

Detection at 271 nm

Results: Single peak at R<sub>t</sub> = 13.41 min