

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

WR-2483

Lot # 7241

The antiserum was raised in a rabbit which was immunized with a peptide analogue of Protein Kinase C Theta (700-706) 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-Theta 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

Polypeptide	% Cross Reactivity
Protein Kinase C Theta	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

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-2482 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 dilution to at least 1:8 (Note: higher dilutions may be needed). Use 2% normal goat serum in TBS/Tween-20 with 2% NGS as buffer for the primary antibody. Let the primary antibody bind for 1 - 2 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-2482 Peptide: Protein Kinase C Theta [Tyr⁶⁹⁹-700-706] (Rat)

Amino Acid Sequence: NH₂-Tyr-Gly-Met-Glu-Arg-Leu-Ile-Ser-COOH

Mol. Wt.: 968.1

Peptide Purity: > 95% (see attached HPLC analysis)

Peptide Quantity: 150 nmoles

Date: March 8, 1994

Lot # 5020

HPLC Analysis

HPLC Analysis: See Chart Recording

Solvent System: A. 0.05 M KH₂PO₄, 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	80	20
	40		40	60
	50		0	100

Detection: optical density at 225 nm

Results: Major peak at R_t = 31.676 min