

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

WR-2416

Lot # 7399

The antiserum was raised in a rabbit which was immunized with a peptide analogue of Protein Kinase C Alpha(664-672) 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-Alpha 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.

<b>Polypeptide</b>	<b>Antiserum Specificity</b>	<b>% Cross Reactivity</b>
Protein Kinase C Alpha		100
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-2416B in 600  $\mu$ l of reconstituted stock antibody. Incubate one hour. Then add 5.4 ml of 1% normal goat serum in TBS-Tween 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.