

**IgG Fraction of Rabbit Anti-Protein Kinase C $\alpha$  [664-672] Serum**

AS-2416G

Lot # 6574

The antiserum was raised in a rabbit which was immunized with synthetic Protein Kinase C $\alpha$  [664-672] covalently attached onto a carrier protein. Rehydrate the lyophilized antiserum with 0.1 ml of 10 mg/ml BSA in PBS for the equivalent of whole antiserum, or with additional buffer for more dilute antiserum. This antiserum has been found to stain specifically PKC $\alpha$  in western immunoblots of whole rat brain homogenates and to stain specifically intracellular regions of fixed cultured cells by indirect immunofluorescence. The antiserum was tested for recognition of other Protein Kinase C isozymes by ELISA techniques.

**Antiserum Specificity**

<b>Polypeptide</b>	<b>% Cross Reactivity</b>
Protein Kinase $\alpha$ [664-672]	100
Protein Kinase C $\beta$ 1 [661-671]	0
Protein Kinase C $\beta$ 2 [660-673]	0
Protein Kinase C $\gamma$ [681-689]	0
Protein Kinase C $\delta$ [662-673]	0
Protein Kinase C $\epsilon$ [728-737]	0
Protein Kinase C $\xi$ [480-492]	0
Protein Kinase C $\eta$	0
Protein Kinase C $\theta$	0
ACTH (human, 1-39)	0
ANP (human)	0
Calcitonin (human)	0
Somatostatin 28	0
Vasoactive Intestinal Peptide	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. Apply the rabbit polyclonal antibody after dilution to at least 1:400 (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.
4. Wash x 3 with TBS/Tween-20.
5. 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.
6. Wash x 4 for 5 minute/wash with TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.

1.