R&D® Ab
Research & Diagnostic Antibodies
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Rabbit Anti-Protein Kinase Cα [664-672] Serum

AS-2416S Lot # 6573

The antiserum was raised in a rabbit which was immunized with synthetic Protein Kinase Cα [664-672] covalently attached onto a carrier protein. Rehydrate the lyophilized antiserum with 0.1 ml of 10 mg/ml BSA is PBS for the equivalent of whole antiserum, or with additional buffer for more dilute antiserum. This antiserum has been found to stain specifically PKCα 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

Polypeptide	% Cross Reactivity
Protein Kinase α [664-672]	100
Protein Kinase Cβ1[661-671]	0
Protein Kinase Cβ2[660-673]	0
Protein Kinase Cγ [681-689]	0
Protein Kinase Cδ [662-673]	0
Protein Kinase Cε [728-737]	0
Protein Kinase Cξ [480-492]	0
Protein Kinase Cη	0
Protein Kinase Cθ	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 at 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.

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