R&D Ab ®

Research & Diagnostic Antibodies

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Rabbit Anti-Human Protein Kinase Cε [728-737] Serum

AS-2453S Lot # 10252

The pooled antisera were raised in rabbits which were immunized with synthetic Protein Kinase Cε [728-737] covalently attached onto a carrier protein. Rehydrate the lyophilized antibody with 0.1 ml of 10 mg/ml BSA in PBS for the equivalent of whole antisera, or with additional buffer for more dilute antisera. This pool of antisera has been found to stain specifically fixed cultured cells by indirect immunofluorescence. The antisera were tested for recognition of other Protein Kinase C isozymes by ELISA techniques.

Antiserum Specificity

Polypeptide	% Cross Reactivity
Protein Kinase Cε [728-737]	100
Protein Kinase Cα [664-672]	0
Protein Kinase Cβ1 [662-671]	0
Protein Kinase Cβ2 [660-673]	0
Protein Kinase Cγ [681-689]	0
Protein Kinase Cδ [662-673]	0
Protein Kinase Cξ [480-492] (rat)	0
Protein Kinase Cη [676-683]	0
Protein Kinase Cθ [700-706]	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 1:500 (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.