R&D ® Ab
Research & Diagnostic Antibodies
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IgG Fraction of Rabbit Anti-Rat Protein Kinase C ε (728-737) Sera

AS-2453G Lot # 5838

The antiserum was raised in a rabbit which was immunized with synthetic Protein Kinase C_ε covalently attached onto a carrier protein, and it has been characterized by immunocytochemical, western immunoblot and ELISA techniques. The antiserum and the IgG fraction of the antiserum have been found to be highly specific for this peptide sequence and are suitable for immunocytochemical and western blot detection of the Protein Kinase C_ε. Rehydrate the lyophilized IgG fraction with 0.1 ml of PBS which contains 10 mg/ml BSA or with additional buffer (such as 10 mg/ml BSA in PBS) for more dilute antibody.

Protein Concentration = 2.5 mg

Antiserum Specificity

Polypeptide	% Cross Reactivity
Protein Kinase C ε	100
Protein Kinase C β1	<0.02
Protein Kinase C α	<0.02
Protein Kinase C δ	<0.01
Protein Kinase C γ	0
Protein Kinase C β2	<0.02
Protein Kinase C ζ	<0.01
Protein Kinase C η	0
Protein Kinase C θ	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 IgG fraction after dilution to at least 40 μg/ml (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.