

Product: PKCeta, Active

Catalog #: 02-2046

Amount: 5 µg

Product Description

Recombinant full-length human PKCeta was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is [NM_006255](#).

Gene Aliases

PKCL; PKC-L; PRKCL; MGC5363; PRKCH

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

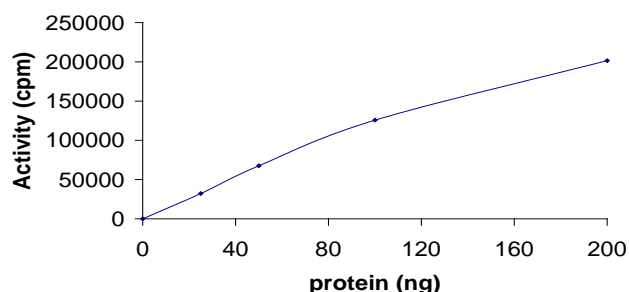
Scientific Background

PKCeta is a member of the protein kinase C (PKC) family of serine- and threonine-specific protein kinases that can phosphorylate a wide variety of protein targets known to be involved in diverse cellular signaling pathways. PKCeta is predominantly expressed in squamous cell epithelia and induces terminal differentiation of keratinocytes. PKCeta that is endogenously expressed or overexpressed is found to associate with the cyclin E/cdk2/p21 complex in keratinocytes of mice and humans (1).

References

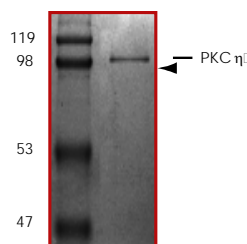
1. Kashiwagi, M. et al: PKCeta associates with cyclin E/cdk2/p21 complex, phosphorylates p21 and inhibits cdk2 kinase in keratinocytes. *Oncogene*. 2000 Dec 14;19(54):6334-41.

Specific Activity



The specific activity of PKCeta was determined to be **86 nmol /min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>90%** by densitometry. Approx. MW **103kDa**.

PKCeta, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	02-2046
Quantity	5µg
Specific Activity	86 nmol/min/mg
Specific Lot Number	B034-1
Purity	>90%
Format	5µg in 50µl
Concentration	0.1µg/µl
Stability	1yr At -70°C from date of shipment
Storage & Shipping	Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

This product is sold for laboratory research use or further manufacturing only and should not be used for human therapeutic or diagnostic applications. The information presented is believed to be accurate; however, said information and products are offered without warranty or guarantee since the ultimate conditions of use and the variability of the materials treated are beyond our control. Nothing disclosed herein is to be construed as a recommendation to use our products in violation of any patents. Under no circumstances shall ARP American Research Products, Inc. be liable for damages, whether consequential, compensatory, incidental or special, strict liability or negligence, breach of warranty or any other theory arising out of the use of the products available from ARP American Research Products, Inc. Nothing contained herein warrants that the use of the products will not infringe on the claims of any patents covering the product itself or the use thereof in combination with other products or in the operation of any process.

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: 02-2046)

Active PKCeta (0.1µg/µl) diluted with Kinase Dilution Buffer (Catalog #: K21-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active PKCeta for optimal results).

Kinase Dilution Buffer, pH 7.2 (Catalog #: K21-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with distilled .

Kinase Assay Buffer I, pH 7.2 (Catalog #: K01-09)

Buffer components: 25mM MOPS, 12.5mM β-glycerol-phosphate, 25mM , 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³²P]-ATP Assay Cocktail

Prepare 250µM [³²P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³²P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer (Catalog #: K01-09). Store 1ml aliquots at –.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer (Catalog #: K01-09). Store 200µl aliquots at –.

Substrate

PKC synthetic peptide substrate (ERM₁PRK₂QGSVRRRV) diluted in distilled to a final concentration of 1mg/ml.

Assay Protocol

- Step 1. Thaw [³²P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2. Thaw the Active PKCeta, Kinase Assay Buffer, Substrate and Enzyme Dilution Buffer on ice.
- Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
 - Component 1. 10µl of diluted Active PKCeta (Catalog #02-2046)
 - Component 2. 7.5µl of 1mg/ml stock solution of substrate
 - Component 3. 2.5µl lipid activator (0.5 mg/ml phosphatidylserine and 0.05 mg/ml diacylglycerol in 20 mM MOPS, pH 7.2, containing 1 mM CaCl₂). Sonicate lipid for 1 minute prior to use.
- Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
- Step 5. Initiate the reaction by the addition of 5µl [³²P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [³²P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [³²P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ³²P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

This product is sold for laboratory research use or further manufacturing only and should not be used for human therapeutic or diagnostic applications. The information presented is believed to be accurate; however, said information and products are offered without warranty or guarantee since the ultimate conditions of use and the variability of the materials treated are beyond our control. Nothing disclosed herein is to be construed as a recommendation to use our products in violation of any patents. Under no circumstances shall ARP American Research Products, Inc. be liable for damages, whether consequential, compensatory, incidental or special, strict liability or negligence, breach of warranty or any other theory arising out of the use of the products available from ARP American Research Products, Inc. Nothing contained herein warrants that the use of the products will not infringe on the claims of any patents covering the product itself or the use thereof in combination with other products or in the operation of any process.