

## Product: PKCzeta, Active

Catalog #: 02-2051

Amount: 5 µg

### Product Description

Recombinant full-length human PKCzeta was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is [NM\\_002744](#).

### Gene Aliases

PRKCZ; PRKCZ

### 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.

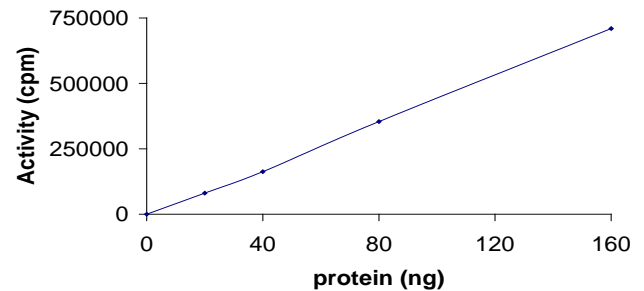
### Scientific Background

PKCzeta is an atypical isoform of the PKC family. PKCzeta is found in both particulate and soluble fractions and cannot be activated by phorbol ester. Overexpression of PKCzeta and subsequent phorbol ester treatment abolished phorbol ester-induced reduction in cell proliferation (1). Overexpression of PKCzeta also potentiates phorbol ester-induced mitogen-activated protein (MAP) kinase activation in a PKC-dependent manner. PKCzeta is an upstream modulator of p70S6K, an important regulator of cell proliferation (2).

### References

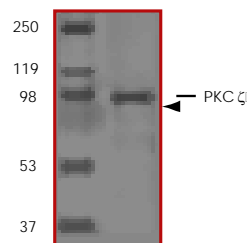
- Kim, S.J. Et al: Phorbol ester effects in atypical protein kinase C zeta overexpressing NIH3T3 cells: possible evidence for crosstalk between protein kinase C isoforms. *Biochem Biophys Res Commun.* 1997 Aug 18;237(2):336-9.
- Romanelli, A. et al: p70 S6 kinase is regulated by protein kinase Czeta and participates in a phosphoinositide 3-kinase-regulated signalling complex. *Mol Cell Biol.* 1999 Apr;19(4):2921-8.

### Specific Activity



The specific activity of PKCzeta was determined to be **114 nmol /min/mg** as per activity assay protocol.

### Purity



The purity was determined to be **>90%** by densitometry. Approx. MW **93kDa**.

## PKCzeta, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	<b>02-2051</b>
Quantity	5µg
Specific Activity	114 nmol/min/mg
Specific Lot Number	B242-1
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.

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## Activity Assay Protocol

### Reaction Components

**Active Kinase** (Catalog #: 02-2051)

Active PKCzeta (0.1µg/µl) diluted with Kinase Dilution Buffer (Catalog #: K23-09) 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 PKCzeta for optimal results).

**Kinase Dilution Buffer, pH 7.2** (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50 ng/µl BSA solution.

**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.

**[γ]-ATP Assay Cocktail**

Prepare 250µM [γ]-ATP Assay Cocktail in a designated radioactive working area by adding the following radioactive components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [<sup>32</sup>P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer (Catalog #: K01-09). Store 1ml aliquots at -20°C.

**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 -20°C.

**Substrate**

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

### Assay Protocol

- Step 1. Thaw [<sup>32</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2. Thaw the Active PKCzeta, 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 PKCzeta (Catalog # 02-2051)
  - Component 2. 10µl of 1mg/ml stock solution of substrate
- 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 H<sub>2</sub>O.
- Step 5. Initiate the reaction by the addition of 5µl [<sup>32</sup>P]-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 H<sub>2</sub>O) 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 [P<sup>32</sup>]-ATP Specific Activity (SA) (cpm/pmol)**

Specific activity (SA) = cpm for 5µl [<sup>32</sup>P]-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 <sup>32</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg)]\*[(Reaction Volume) / (Spot Volume)]

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