PKC<sub>nu</sub>, Active
Full-length human recombinant protein expressed in Sf9 cells

Catalog # P73-10G
Lot # X393-2

Product Description

Recombinant full-length human PKC<sub>v</sub> was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_005813.

Gene Aliases
EPK2; PRKCN

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 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

PKC<sub>v</sub>, also known as PKD3, is a member of the protein kinase C (PKC) family of serine/threonine kinases that play critical roles in the regulation of cellular differentiation and proliferation in many cell types. PKC<sub>v</sub> is composed of 890 amino acid residues and the protein has 77.3% similarity to human PKC mu (PKC<sub>µ</sub>) and 77.4% similarity to mouse PKD (1). The PKC<sub>v</sub> mRNA is ubiquitously expressed in various tissues and the gene is located between markers WI-9798 and D2S177 on chromosome 2p21 region.

References
Activity Assay Protocol

Reagents and Materials

Active Kinase (Catalog #: P73-10G)
Active PKC (0.1 µg/µl) diluted with Kinase Dilution Buffer III (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 PKC for optimal results).

Kinase Dilution Buffer III (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 (Catalog #: K01-09)
Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgC12, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[3³P]-ATP Assay Cocktail
Prepare 250µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³³P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at –20°C.

10M ATP Stock Solution (Catalog #: A50-09)
Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200µl aliquots at –20°C.

Substrate (Catalog #: C50-58)
CREBlide synthetic peptide substrate (KRREILSRRPSYR) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
Step 2. Thaw the Active PKC, Kinase Assay Buffer, Substrate and Kinase 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 PKC (Catalog #P73-10G)
Component 2. 5µl of 1mg/ml stock solution of substrate (Catalog #C50-58)
Component 3. 5µl distilled H₂O (4°C)

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 [³³P]-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 [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [³³P]-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 ³³P-ATP in cpm/pmol]*[Reaction time in min]*[Enzyme amount in µg or mg]) / ([Reaction Volume) / (Spot Volume)]