

Product Specification

PKC ν , active

(Full-length recombinant protein expressed in Sf 9 cells)

Catalog #: 7746-5
 Lot #: _____
 Aliquot size: 5 μ g protein in 50 μ l
 Specific activity: 177 nmol/min/mg

Quality Control Analysis

Activity assessment

PKC ν protein (~100 ng/ μ l concentration) was diluted to 50ng/ μ l with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β -glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM MgCl₂, 0.05 mM DTT and 40ng/ \square l BSA), followed by 2-fold serial dilutions, and then the 10 μ l diluted proteins were used to phosphorylate the CREBTIDE substrate peptide (KRREILSRRPSYR) in the following assay condition:

- 10 μ l diluted PKC ν protein
- 10 μ l CREBTIDE substrate peptide (1 mg/ml stock)
- 5 μ l [³²P] ATP mixture (250 μ M ATP, 0.16 μ Ci/ μ l in 4x assay dilution buffer)

The various reaction components, except [³²P] ATP, were incubated at 30⁰C and the reaction started by the addition of [³²P] ATP. After 15 minutes, the reaction was terminated by spotting 20 μ l of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.

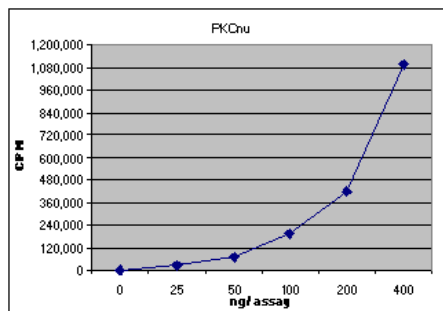


Fig. 1 PKC ν activity assay

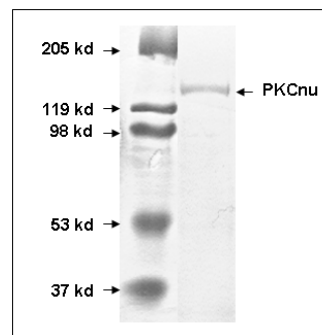


Fig. 2 PKC ν protein gel

Purity assessment

1 μ g of PKC ν protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the blue gel showed >80% purity of the PKC ν protein product, and the band was at ~142 kDa (Fig. 2).

Product Description

Recombinant full length human PKCnu containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.

The gene accession number is NM_005813.

This material is sold for research purposes only.

Specific Activity

177 nmol phosphate incorporated into CREBTIDE substrate peptide per minute per mg protein at 30°C for 15 minutes using a final concentration of 50 μM ATP (0.83 μCi/assay).

Formulation

Recombinant proteins in storage buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol).

Storage and Stability

Store product frozen at or below -70°C. Stable for 1 year at -70°C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

Scientific Background

PKCnu, 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. PKCnu is composed of 890 amino acid residues and the protein has 77.3% similarity to human PKC mu (PKCmu) and 77.4% similarity to mouse PKD (the mouse homolog of PKCmu) (1). The PKCnu mRNA is ubiquitously expressed in various tissues and the gene is located between markers WI-9798 and D2S177 on chromosome 2p21 region.

PKCnu has two putative diacylglycerol binding C1 domains, suggesting that it may participate in a novel diacylglycerol-mediated signaling pathway (2). PKCnu is trans-located to the plasma membrane and activated by the diacylglycerol mimic phorbol 12-myristate 13-acetate. PKCnu is an important physiologic target of the B-cell receptor (BCR) and exhibits robust activation after BCR engagement (2). GPCR agonists induced a rapid activation of PKCnu by a protein kinase C (PKC)-dependent pathway that leads to the phosphorylation of the activation loop of PKCnu. PKCnu is present both in the nucleus and cytoplasm and this distribution of PKCnu results from its continuous shuttling between both compartments by a mechanism that requires a nuclear import receptor and a competent CRM1-nuclear export pathway (3). Cell stimulation with the GPCR agonist neurotensin induced a rapid and reversible plasma membrane translocation of PKCnu that is PKC-dependent.

References

1. Hayashi A, Seki N, Hattori A, Kozuma S, Saito T. PKCnu, a new member of the protein kinase C family, composes a fourth subfamily with PKCmu. *Biochim Biophys Acta*. 1999 May 6;1450(1):99-106.
2. Matthews SA, Dayalu R, Thompson LJ, Scharenberg AM. Regulation of protein kinase Cnu by the B-cell antigen receptor. *J Biol Chem*. 2003 Mar 14;278(11):9086-91.
3. Rey O, Yuan J, Young SH, Rozengurt E. Protein kinase C nu/protein kinase D3 nuclear localization, catalytic activation, and intracellular redistribution in response to G protein-coupled receptor agonists. *J Biol Chem*. 2003 Jun 27;278(26):23773-85.