

Product Specification

PKC iota, active

(Full-length recombinant protein expressed in Sf9 cells)

Catalog # : 7705-5
 Lot #: _____
 Aliquot size: 5 µg protein in 50 µl
 Specific activity: 664 nmol/min/mg

Quality Control Analysis

Activity assessment

PKC iota protein (0.1 µg/µl concentration) was diluted to 26.7ng/µl 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), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the CREBtide (KRREILSRPSYR) in the following assay condition:

7.5 µl	diluted PKC iota protein
10 µl	CREBtide (1 mg/ml stock)
2.5 µl	lipid activators (0.5 mg/ml phosphatidylserine and 0.05 mg/ml diacylglycerol in 20 mM MOPS, pH 7.2, 25 mM β-glycerophosphate, 1 mM sodium orthovanadate, 1 mM dithioereitol, 1 mM CaCl ₂). Sonicate for 1 minute prior to use.
5 µl	[³² P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [³²P] ATP, were incubated at 30°C and the reaction started by the addition of [³²P] ATP. After 15 minutes, the reaction was terminated by spotting 20 µl 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.

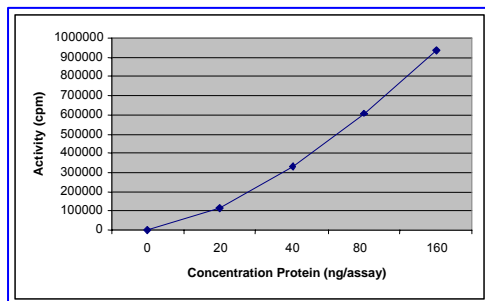


Fig. 1 PKC iota activity assay

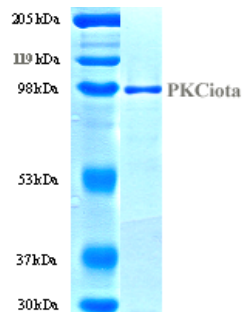


Fig. 2 PKC iota protein gel

Purity assessment

1 µg of PKC iota protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the PKC iota product, and the band was at ~98 kDa (Fig. 2).

Product Description

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

The gene accession number is NM_002740.

This material is sold for research purposes only.

Specific Activity

664 nmol phosphate incorporated into CREBtide per minute per mg protein at 30°C for 15 minutes using a final concentration of 50 μ M ATP (0.83 μ Ci/assay in 25ul reaction volume).

Formulation

Recombinant protein 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

PKC iota is a member of the protein kinase C family of serine-threonine kinases. The amino acid sequence of PKC iota showed greatest homology to PKC zeta, with 72% identity overall rising to 84% in the catalytic domain. In contrast, the homology of PKC iota to the other isoforms is less pronounced, with < 53% identity even in the highly conserved catalytic region. PKC iota transcript is present predominantly in lung and brain, but also expressed at lower levels in many tissues including pancreatic islets. PKC iota is stimulated by tumor necrosis factor alpha (TNF- α) and is required for the activation of NF- κ B by this cytokine. Cell transfections with a PKC iota dominant negative mutant abolished TNF- α -induced NF κ B-dependent transcription. PKC iota can modify vulnerability of neural cells to apoptosis induced by amyloid beta-peptide (ABP), a cytotoxic peptide linked to neuronal degeneration in Alzheimer's disease. Associated with the increased resistance to apoptosis are improved mitochondrial function and reduced activity of caspases. In addition, ABP-induced increases in levels of oxidative stress and intracellular calcium levels were attenuated in cells overexpressing PKC iota.