

## Product Specification

### **PKC $\mu$ , active**

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

Catalog #: 7745-5  
Lot #: \_\_\_\_\_  
Aliquot size: 5  $\mu$ g protein in 50  $\mu$ l  
Specific activity: 658 nmol/min/mg

### **Quality Control Analysis**

#### Activity assessment

PKC $\mu$  protein (~100 ng/ $\mu$ l concentration) was diluted to 20ng/ $\mu$ l with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM  $\beta$ -glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM MgCl<sub>2</sub>, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10 $\mu$ l diluted proteins were used to phosphorylate the CREBTIDE substrate peptide (KRREILSRPSYR) in the following assay condition:

- 10  $\mu$ l diluted PKC $\mu$  protein
- 10  $\mu$ l CREBTIDE substrate peptide (1 mg/ml stock)
- 5  $\mu$ l [<sup>32</sup>P] ATP mixture (250  $\mu$ M ATP, 166 nCi/ $\mu$ l in 4x assay dilution buffer)

The various reaction components, except [<sup>32</sup>P] ATP, were incubated at 30<sup>0</sup>C and the reaction started by the addition of [<sup>32</sup>P] ATP. After 15 minutes, the reaction was terminated by spotting 20  $\mu$ 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.

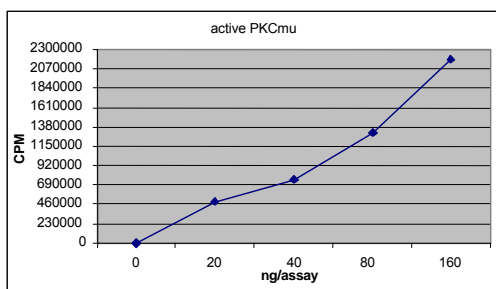


Fig. 1 PKC $\mu$  activity assay

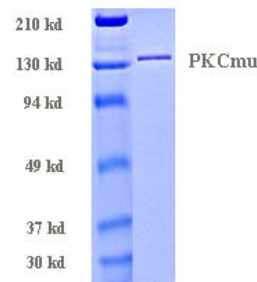


Fig. 2 PKC $\mu$  protein gel

#### Purity assessment

1  $\mu$ g of PKC $\mu$  protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the PKC $\mu$  product, and the band was at ~131 kDa (Fig. 2).

### **Product Description**

Recombinant full length human PKC $\mu$  containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells. The gene accession number is X75756.

This material is sold for research purposes only.

### Specific Activity

658 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

Protein kinase Cmu (PKCmu) is a novel member of the protein kinase C (PKC) family that differs from the other isoenzymes in structural and enzymatic properties. It is characterized by the presence of a pleckstrin homology (PH) domain and an amino-terminal hydrophobic region and has a substrate specificity distinct from other PKC isoforms. PKCmu is a ubiquitous PKC isotype with the highest expression in the thymus, lung and peripheral blood mononuclear cells (1). PKCmu forms a complex in vivo with a phosphatidylinositol 4-kinase and a phosphatidylinositol-4-phosphate 5-kinase. A region of PKCmu between the amino-terminal transmembrane domain and the pleckstrin homology domain is shown to be involved in the association with the lipid kinases (2). PKCmu was also shown to associate with the B cell receptor (BCR) complex and its activity is up-regulated after cross-linking the BCR and CD19 on B cells (3). PKC mu co-precipitates with Syk and phospholipase C-gamma 1/2 (PLC gamma 1/2) and in vitro phosphorylation of fusion proteins showed that both Syk and PLC gamma 1 are potential substrates of PKC mu in vivo. In addition, specific interaction of PKCmu and 14-3-3tau can be shown in the T cell line Jurkat by immunoprecipitation and by pulldown assays (4). 14-3-3tau is not a substrate of PKCmu and strongly down-regulates PKCmu kinase activity in vitro. In response to various stimuli, PKC mu activates the mitogen-activated protein kinase (p42/ERK1 MAPK cascade) but does not affect the related c-jun N-terminal kinase nor p38 MAPK (5).

### References

1. Rennecke J, Johannes FJ, Richter KH, Kittstein W, Marks F, Gschwendt M. *Immunological demonstration of protein kinase C mu in murine tissues and various cell lines. Differential recognition of phosphorylated forms and lack of down-regulation upon 12-O-tetradecanoylphosphol-13-acetate treatment of cells.* Eur J Biochem. 1996 Dec 1;242(2):428-32.
2. Nishikawa K, Toker A, Wong K, Marignani PA, Johannes FJ, Cantley LC. *Association of protein kinase Cmu with type II phosphatidylinositol 4-kinase and type I phosphatidylinositol-4-phosphate 5-kinase.* J Biol Chem. 1998 Sep 4;273(36):23126-33.
3. Sidorenko SP, Law CL, Klaus SJ, Chandran KA, Takata M, Kurosaki T, Clark EA. *Protein kinase C mu (PKC mu) associates with the B cell antigen receptor complex and regulates lymphocyte signaling.* Immunity. 1996 Oct;5(4):353-63.
4. Hausser A, Storz P, Link G, Stoll H, Liu YC, Altman A, Pfizenmaier K, Johannes FJ. *Protein kinase C mu is negatively regulated by 14-3-3 signal transduction proteins.* J Biol Chem. 1999 Apr 2;274(14):9258-64.
5. Hausser A, Storz P, Hubner S, Braendlin I, Martinez-Moya M, Link G, Johannes FJ. *Protein kinase C mu selectively activates the mitogen-activated protein kinase (MAPK) p42 pathway.* FEBS Lett. 2001 Mar 9;492(1-2):39-44.