3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-521-8956 • (314) 771-5765
Fax 800-325-5052 • (314) 771-5757
sigma-aldrich.com

ProductInformation

PROTEIN KINASE C EPSILON ISOZYME
Human, Recombinant

Product Code **P 1164**
Storage Temperature –70 °C

Synonym: PKC Epsilon

Product Description

This product is human recombinant protein produced by Baculovirus-mediated expression in insect cells. This protein is purified to near homogeneity (greater than 95% by SDS-PAGE) and, therefore, may behave differently from crude preparations. The calculated molecular weight is 83.5 kDa, but the apparent molecular weight is 89-96 kDa.[1]

Protein Kinase C (PKC) is a serine/threonine kinase first characterized by Nishizuka[2] on the basis of its activation *in vitro* by Ca^{2+}, phospholipid (primarily phosphatidylserine), and diacylglycerol (DAG). PKC is activated intracellularly by signal transduction pathways that produce DAG along with some lysophospholipids and fatty acids, from phosphatidylinositol diphosphate (PIP2) and phosphatidylcholine (PC) through the action of various activated phospholipases. Phorbol ester can also stimulate PKC, probably by a mechanism similar to that used by DAG and has, therefore, been a useful tool in the study of PKC.

PKC plays an important role in the regulation of diverse cellular functions. In humans at least 11 different PKC polypeptides have been identified. These isoforms can be grouped into three subfamilies and include alpha, beta I, beta II, gamma, delta, epsilon, zeta, eta, theta, mu, and iota. These isoforms differ in primary structure, tissue distribution, subcellular localization, mode of action in vitro, response to extracellular signals, and substrate specificity.[3] PKC : alpha, beta I, beta II, gamma form the first family and their activities are Ca^{2+} and phospholipid-dependent, while delta, epsilon, eta, and theta PKC comprise the second family and are Ca^{2+}-independent, but phospholipid-dependent. PKC zeta, mu, and iota form the third family and are not activated by phorbol esters or DAG.

Vial contents: a solution in 20 mM HEPES, pH 7.4; 2 mM EDTA, 2 mM EGTA, 5 mM DTT, 100 mM NaCl, 0.05% Triton X-100, and 50% glycerol.

Preparation Instructions

Dilutions can be made in 10 mM HEPES (pH 7.4), 5 mM DTT, 0.01% Triton X-100.

Storage/Stability

Store at –70 °C.

Procedure

Materials Required but Not Supplied:
0.5 M HEPES (pH 7.4)
1 mM EGTA
100 mM $MgCl_2$
PKC Epsilon substrate peptide
(ERMRPRKRQGSVRRRV)
10 mM ATP
[γ^{32}-P]ATP
10 mg/ml phospatidylserine (PS), (Product No. P 6641)
2 mg/ml diacylglycerol (DAG), (Product No. D 0138)
Triton X-100
Dithiothreitol (DTT)
5% phosphoric acid
phosphocellulose membrane
Hamilton syringe

Lipid Mix Preparation

Each reaction requires 12 µg of PS (1.2 µl of 10 mg/ml PS Stock) and 1.2 µg of DAG (0.6 µl of 2 mg/ml DAG Stock).

1. Determine the total amount of each reagent for the number of reactions to be performed and make up 10% more lipid mix than required to account for pipetting losses.
2. Using a Hamilton syringe that has been cleaned out with methanol, transfer the required volume of each lipid stock to a 12 x 75 mm glass test tube.
3. Thoroughly dry the chloroform with a nitrogen stream while gently rotating the tube.

4. Resuspend the dried mixture in 6 µl of lipid resuspension buffer per reaction. Resuspension buffer is 10 mM HEPES (pH 7.4), 0.3% Triton X-100. Vortex into suspension. This will take at least 2 minutes of vortexing.
5. Place the lipid mix in a 40°C water bath for 5 minutes prior to adding it to the reaction mix.

Activity Assay

All assays should be performed in triplicate. In addition, include two blanks (reaction mix with no enzyme added).

1. Prepare the reaction mix as follows:

Vol.	Reagent	Final Conc.
2.4 µl	0.5M HEPES (pH 7.4)	20 mM
6 µl	100 mM $MgCl_2$	10 mM
6 µl	1 mM EGTA	0.1 mM
6 µl	1 mg/ml substrate peptide	100 µg/ml
0.6 µl	10 mM ATP	100 µM
6 µl	Lipid Mix (see above)	*
0.1 µl	[γ^{32}-P]ATP**	trace
32.9 µl	distilled water	------
Total = 60 µl		

* Final conc: 200 µg/ml PS, 20 µg/ml DAG, 1 mM HEPES, 0.03% Triton X-100
**Add more if isotope is over one week old.

Determine the total amount of each reagent required for the number of reactions to be performed and make up 10% more reaction mix than required to account for pipetting losses.

2. Dispense 60 µl of the reaction mix into each assay tube and place the tubes at 30 °C.
3. Dilute the enzyme to be assayed to a final concentration of 20-50 ng/µl, using dilution buffer (10 mM HEPES, pH 7.4, 5 mM DTT, 0.01% Triton X-100).

Note: Since it is difficult to make accurate dilutions when pipetting small volumes (<5 µl), we recommend using at least 5 µl of enzyme in the dilution. Example: For a 1:100 dilution, add 5 µl of enzyme to 495 µl of dilution buffer.

4. Add 2 µl of diluted enzyme to each assay tube at 20-second intervals. For blanks, add 2 µl of dilution buffer instead of diluted enzyme.
5. Stop the reactions after 10 minutes by adding 6 µl of 5% phosphoric acid to each assay tube (including blanks).
6. Incubate on ice for 5 minutes.
7. Transfer 50 µl from each assay tube to phosphocellulose membranes. Allow to dry.
8. Wash the membranes 3 times with 5 ml of 0.5% phosphoric acid per filter in a 400 ml beaker.
9. In addition, spot 5 µl of the reaction mix (from step 1) onto two phosphocellulose membranes. These samples will be used to determine total cpm in a reaction.
10. Transfer all membranes to scintillation vials and count. It is not necessary to dry the membranes before counting.

One unit is defined as the amount of enzyme necessary to transfer 1 nmol of phosphate to the PKC epsilon substrate peptide in 1 minute at 30 °C at pH 7.4.

See formula for activity calculation at the bottom of this document.

References

1. Hug, H., and Sarre, T.F., Biochem. J., **291,** 329-343 (1993).
2. Nishizuka, Y., Science, **233,** 305-312 (1986).
3. Jaken, S., Current Opinion in Cell Biology, **8,** 168-173 (1996).
4. Kazanietz, M.G., et al., Mol. Pharmacol., **44,** 298-307 (1993).
5. Newton, A.C., J. Biol. Chem., **270,** 28495-28498 (1995).
6. Epand, R.M., Anal. Biochem., **218,** 241-247 (1995).
7. Dekker, L.V., and Parker, P.J., Trends Biochem. Sci., **19,** 73-77 (1994).
8. Nishizuka, Y., Science, **258,** 607-614 (1992).
9. Asaoka, Y., et al., Trends Biochem. Sci., **17,** 414-417 (1992).
10. Bell, R.M., and Burns, D.J., J. Biol. Chem., **266,** 4661-4664 (1991).

PKC Isozyme Reference Guide						
Isoform	**Type**	**Calcium Dependent**	**Phorbol stimulation**	**Predicited MW**	**Apparent MW**	**Suggested Substrates**
Alpha	Conventional	Yes	Yes	76.8 kDa	80-81 kDa	alpha pseudosubstrate peptide, Histone H3
beta I	Conventional	Yes	Yes	76.8 kDa	79-80 kDa	alpha pseudosubstrate peptide, Histone H3
beta II	Conventional	Yes	Yes	76.9 kDa	80 kDa	alpha pseudosubstrate peptide, Histone H3
gamma	Conventional	Yes	Yes	78.4 kDa	77-84 kDa	alpha pseudosubstrate peptide, Histone H3
delta	Novel	No	Yes	77.5 kDa	74-79 kDa	alpha and epsilon pseudosubstrate peptides
epsilon	Novel	No	Yes	83.5 kDa	89-96 kDa	alpha and epsilon pseudosubstrate peptides
eta	Novel	No	Yes	77.9 kDa	82-84 kDa	alpha and epsilon pseudosubstrate peptides
zeta	Atypical	No	No	67.7 kDa	76-80 kDa	alpha and epsilon pseudosubstrate peptides

Activity Calculation:

$$\text{units}/\mu l = \frac{(\text{cpm sample} - \text{cpm blank}) \times (\text{dilution factor}) \times [\text{total assay vol.} \div \text{vol. spotted}] \times [\text{nm ATP added} \div \text{total cpm}]}{(\mu l \text{ enzyme added}) \times \text{assay time in minutes}}$$

$$\text{where total cpm in assay} = \frac{\mathbf{X} \text{ cpm (see step 9)}}{5\ \mu l \text{ reaction mix}} \times 60\ \mu l \text{ reaction mix/assay}$$

Note: Specific Activity (units/mg) may be calculated by dividing the units/µl by the protein concentration.

AC/EWK 12-19-00