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# ProductInformation

**Protein Kinase C, Zeta Isozyme**
**human, recombinant**
**expressed in insect cells**

Product Code **P 0194**
Storage Temperature –70 °C

Synonym: PKC Zeta; Protein Kinase C, ζ Isozyme

**Product Description**
This product is the human recombinant protein produced by baculovirus-mediated expression in insect cells. The calculated molecular weight is 67.7 kDa, but the apparent molecular weight is 76-80 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 isoforms have been identified, that 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, and 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.

The product is supplied in a solution of 20 mM HEPES, pH 7.4, 2 mM EDTA, 2 mM EGTA, 5 mM DTT, 250 mM NaCl, 0.05% TRITON™ X-100, and 50% glycerol.

**Preparation Instructions**
Dilutions can be made in 10 mM Tris-HCl, pH 7.5, with 5 mM DTT and 0.01% TRITON X-100.

**Storage/Stability**
Store the product at –70 °C.

**Procedure**
Materials Required but Not Supplied:
1 M Tris-HCl, pH 7.5
50 mM EGTA
100 mM $MgCl_2$
PKC Epsilon substrate peptide
(ERMRPRKRQGSVRRRV)
10 mM ATP
[$\gamma$-$^{32}$P] ATP
10 mg/ml phosphatidylserine (PS),
(Product Code P 6641)
TRITON X-100
Dithiothreitol (DTT)
5% phosphoric acid
phosphocellulose membrane
Hamilton® syringe

Lipid Mix Preparation
10 µg of phosphatidylserine (PS) are needed per reaction.

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 with methanol, transfer the required volume of 10 mg/ml PS 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 10 µl of lipid resuspension buffer per reaction. The resuspension buffer is 10 mM Tris-HCl, pH 7.5. Vortex into solution/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. |
|---|---|---|
| 2.5 µl | 1 M Tris-HCl, pH 7.5 | 25 mM |
| 5 µl | 100 mM $MgCl_2$ | 5 mM |
| 1 µl | 50 mM EGTA | 0.5 mM |
| 1 µl | 100 mM DTT | 1 mM |
| 10 µl | 1 mg/ml substrate peptide | 100 µg/ml |
| 1 µl | 10 mM ATP | 100 µM |
| 10 µl | Lipid Mix (see above) | * |
| 0.1 µl | [$\gamma$-$^{32}$P]ATP** | trace |
| 69.4 µl | distilled water | ------ |
| Total = 100 µl | | |

*Final conc: 100 µg/ml PS, 1 mM Tris-HCl, pH 7.5
**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 100 µl of reaction mix into each assay tube and place tubes at 30 °C.

3. Dilute the protein to be assayed to a final concentration of 20-50 ng/µl using the dilution buffer (10 mM Tris-HCl, pH 7.5, with 5 mM DTT and 0.01% Triton X-100).
   Note: Since it is difficult to make accurate dilutions when pipetting small volumes (<5 µl), it is recommended to use at least 5 µl of enzyme in the dilution. Example: For a 100-fold 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 10 µl of 5% phosphoric acid to all tubes (including blanks).

6. Incubate on ice for 5 minutes.

7. Transfer 11 µl from each assay tube to phosphocellulose membranes. Allow to dry.

8. Wash the membranes 3 times with 50 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 the membranes to scintillation vials and count. It is not necessary to dry the membranes before counting.

11. Calculate enzyme activity.

Activity Calculation:

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

$$\text{where total cpm in assay} = \frac{\mathbf{X}\ \text{cpm (see step 9)} \times 100\ \text{µl reaction mix/assay}}{5\ \text{µl reaction mix}}$$

One unit is defined as the amount of enzyme necessary to transfer 1 nmole of phosphate to the PKC epsilon substrate peptide in 1 minute at 30 °C at pH 7.5.

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

| 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 |

**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).




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