3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Protein kinase C, βII Isozyme
human, recombinant
expressed in baculovirus infected insect cells

Catalog Number **P3287**
Storage Temperature –70 °C

Synonym: PKC Beta II

Product Description
Protein Kinase C (PKC) is a serine/threonine kinase first characterized by Nishizuka[1] 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.[2] 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 (see Table 1).

This product is a human recombinant protein produced by baculovirus-mediated expression in insect cells. It is supplied in a solution of 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.

Purity: >85% (SDS-PAGE)

Calculated molecular mass: 76.9 kDa
Apparent molecular mass:[3] 80 kDa

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

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

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

Procedure
Materials Required but Not Supplied:
0.5 M HEPES, pH 7.4
100 mM $MgCl_2$
10 mM $CaCl_2$
2 mg/ml Histone
10 mM ATP
[γ^{32}-P] ATP
10 mg/ml phosphatidylserine (PS), (Catalog Number P6641)
2 mg/ml diacylglycerol (DAG), (Catalog Number D0138)
Triton X-100
Dithiothreitol (DTT)
1% BSA
TCA
Nitrocellulose membrane
Hamilton syringe

Lipid Mix Preparation

Each reaction requires 10 μg of PS (1.0 μl of 10 mg/ml PS Stock) and 2 μg of DAG (1 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 × 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. 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.
4 μl	0.5 M HEPES, pH 7.4	20 mM
10 μl	100 mM $MgCl_2$	10 mM
1 μl	10 mM $CaCl_2$	0.1 mM
10 μl	2 mg/ml Histone	200 μg/ml
1 μl	10 mM ATP	100 μM
10 μl	Lipid Mix (see above)	*
0.1 μl	[γ^{32}-P]ATP**	trace
63.9 μl	distilled water	–
Total = 100 μl		

* Final conc: 100 μg/ml PS, 20 μg/ml DAG, 1 mM HEPES, and 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 100 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), 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 20 μl of 1% BSA followed immediately by 1 ml of 10% TCA to each assay tube (including blanks).
6. Incubate on ice for 5 minutes.
7. Transfer the contents of each tube to a nitrocellulose membrane on an aspirator funnel. Wash the tube with 1 ml of 5% TCA and add this to the membrane.
8. Wash the membranes with 2 ml of 5% TCA.
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 nmole of phosphate to histones in 1 minute at 30 °C at pH 7.4.

Results
Activity Calculation:

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

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

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

Table 1.
PKC Isozyme Reference Guide

Isoform	Type	Calcium Dependent	Phorbol stimulation	Predicted MW	Apparent MW	Suggested Substrates
alpha	Conventional	Yes	Yes	76.8 kDa	80-81 kDa	alpha pseudosubstrate peptide, Histone
beta I	Conventional	Yes	Yes	76.8 kDa	79-80 kDa	alpha pseudosubstrate peptide, Histone
beta II	Conventional	Yes	Yes	76.9 kDa	80 kDa	alpha pseudosubstrate peptide, Histone
gamma	Conventional	Yes	Yes	78.4 kDa	77-84 kDa	alpha pseudosubstrate peptide, Histone
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

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