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ProductInformation

Protein Kinase C Isozyme Panel
human, recombinant

Product Code **P 6862**
Storage Temperature –70 °C

Synonym: PKC Control Panel

Product Description

The Protein Kinase C Isozyme Panel contains nine isozymes of protein kinase C. Each isozyme is a human recombinant protein produced by Baculavirus-mediated expression in insect cells. These proteins are purified to near homogeneity (greater than 95% by SDS-PAGE, except for eta and theta which are purified to >90%) and may, therefore, behave differently from the corresponding crude preparations. The molecular weight of each isozyme is listed in the table below.

Product Code	Calculated M.W.	Apparent M.W.
P 1782 (alpha - α)	76.8 kDa	80-81 kDa
P 1787 (beta I - βI)	76.8 kDa	79-80 kDa
P 3287 (beta II - βII)	76.9 kDa	80 kDa
P 9542 (gamma - γ)	78.4 kDa	77-84 kDa
P 8538 (delta - δ)	77.5 kDa	74-79 kDa
P 1164 (epsilon - ε)	83.5 kDa	89-96 kDa
P 0194 (zeta - ζ)	67.7 kDa	76-80 kDa
P 3119 (theta - θ)	88.4 kDa	84 kDa
P 0540 (eta - η)	77.9 kDa	82-84 kDa

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 isozymes have been identified. They differ in primary structure, tissue distribution, subcellular localization, *in vitro* mode of action, response to extracellular signals, and substrate specificity.[3]

The isozymes, designated alpha, beta I, beta II, gamma, delta, epsilon, zeta, eta, theta, mu, and iota, can be grouped into three subfamilies. PKC-alpha, -beta I, -beta II, and -gamma form the first family and their activities are Ca^{2+}- and phospholipid-dependent. The second family is comprised of PKC-delta, -epsilon, -eta, and -theta and these 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.

Each isozyme is supplied as a solution in 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

Prepare stock solutions in 10 mM HEPES, pH 7.4, 5 mM DTT, 0.01% TRITON X-100.

Storage/Stability

Store at –70°C. Avoid freeze/thaw cycles. On initial opening store remainder in frozen aliquots at –70 °C.

Assay Procedure

P1782, P1787, P3287, and P9542

Materials Required but Not Supplied:

HEPES
$MgCl_2$
$CaCl_2$
Histone (Product No. H 4524)
Cold ATP
[γ-^{32}P] ATP
Phosphatidylserine (PS) (Product No. P 6641)
Diacylglycerol (DAG) (Product No. D 0138)
TRITON X-100
Dithiothreitol (DTT)
BSA
Trichloroacetic acid (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 washed with methanol, transfer the required volume of each lipid stock to a 12 x 75 mm glass test tube.
3. Thoroughly evaporate the chloroform with a nitrogen stream, while gently rotating the tube.
4. Resuspend the dried mixture in 10 μl of lipid resuspension buffer/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 the reaction mix to it.

Activity Assay

All assays should be performed in triplicate. Include two blanks (reaction mix with no enzyme added).

6. 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	------

100 μl = Total volume

*Final conc: 100 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.

7. Dispense 100 μl of the reaction mix into each assay tube and place the tubes at 30 °C.
8. 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: It is difficult to make accurate dilutions when pipetting small volumes (<5 μl), so 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.
9. 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.
10. 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).
11. Incubate on ice for 5 minutes.
12. 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.
13. Wash the membranes with 2 ml of 5% TCA.
14. To determine total cpm in a reaction spot 5 μl of the reaction mix (from step 6) onto two phosphocellulose membranes.
15. 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 histone in 1 minute at 30 °C at pH 7.4.

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

Assay Procedure
P8538, P1164, P0194, P0540, and P3119

Materials Required but Not Supplied:

HEPES
EGTA
$MgCl_2$
PKC Epsilon substrate peptide:
(ERMRPRKRQGSVRRRV)
Cold ATP
[γ-^{32}P]ATP
Phospatidylserine (PS), (Product No. P 6641)
Diacylglycerol (DAG), (Product No. D 0138)
TRITON X-100
Dithiothreitol (DTT)
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 washed with methanol, transfer the required volume of each lipid stock to a 12 x 75 mm glass test tube.
3. Thoroughly evaporate 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 the reaction mix to it.

Activity Assay

All assays should be performed in triplicate. Include two blanks (reaction mix with no enzyme added).

6. Prepare the reaction mix as follows:

Vol.	Reagent	Final Conc.
2.4 μl	0.5 M 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.

7. Dispense 60 μl of the reaction mix into each assay tube and place the tubes at 30 °C.
8. 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: It is difficult to make accurate dilutions when pipetting small volumes (<5 μl), so 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.
9. 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.
10. Stop the reactions after 10 minutes by adding 6 μl of 5% phosphoric acid to each assay tube (including blanks).
11. Incubate on ice for 5 minutes.
12. Transfer 50 μl from each assay tube to phosphocellulose membranes. Allow to dry.
13. Wash the membranes 3 times with 5 ml of 0.5% phosphoric acid per filter in a 400 ml beaker.
14. To determine total cpm in a reaction spot 5 μl of the reaction mix (from step 6) onto two phosphocellulose membranes.
15. 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

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5. Newton, A. C., Protein kinase C: structure, function, and regulation. J. Biol. Chem., **270,** 28495-28498 (1995).
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7. Dekker, L. V., and Parker, P. J., Protein kinase C—a question of specificity. Trends Biochem. Sci., **19,** 73-77 (1994).
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9. Asaoka, Y., et al., Protein kinase C, calcium and phospholipid degradation. Trends Biochem. Sci., **17,** 414-417 (1992).
10. Bell, R. M., and Burns, D. J., Lipid activation of protein kinase C. 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
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
theta	Novel	No	Yes	84.4 kDa	84 kDa	Alpha and epsilon pseudosubstrate peptides
zeta	Atypical	No	No	67.7 kDa	76-80 kDa	Alpha and epsilon pseudosubstrate peptides

Activity Calculation:

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

$$\text{where total cpm in assay} = \frac{\mathbf{X}\ \text{cpm (see step 14)} \times 100\ \mu\text{l reaction mix/assay}}{5\ \mu\text{l reaction mix}}$$

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

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