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 Baculovirus-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.

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Calculated M.W.</th>
<th>Apparent M.W.</th>
</tr>
</thead>
<tbody>
<tr>
<td>P 1782 (α)</td>
<td>76.8 kDa</td>
<td>80-81 kDa</td>
</tr>
<tr>
<td>P 1787 (βI)</td>
<td>76.8 kDa</td>
<td>79-80 kDa</td>
</tr>
<tr>
<td>P 3287 (βII)</td>
<td>76.9 kDa</td>
<td>80 kDa</td>
</tr>
<tr>
<td>P 9542 (γ)</td>
<td>78.4 kDa</td>
<td>77-84 kDa</td>
</tr>
<tr>
<td>P 8538 (δ)</td>
<td>77.5 kDa</td>
<td>74-79 kDa</td>
</tr>
<tr>
<td>P 1164 (ε)</td>
<td>83.5 kDa</td>
<td>89-96 kDa</td>
</tr>
<tr>
<td>P 0194 (ζ)</td>
<td>67.7 kDa</td>
<td>76-80 kDa</td>
</tr>
<tr>
<td>P 3119 (θ)</td>
<td>88.4 kDa</td>
<td>84 kDa</td>
</tr>
<tr>
<td>P 0540 (η)</td>
<td>77.9 kDa</td>
<td>82-84 kDa</td>
</tr>
</tbody>
</table>

Protein Kinase C (PKC) is a serine/threonine kinase first characterized by Nishizuka on the basis of its activation in vitro by Ca²⁺, phospholipid (primarily phosphatidylinserine), 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.

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 Ca²⁺- and phospholipid-dependent. The second family is comprised of PKC-delta, -epsilon, -eta, and -theta and these are Ca²⁺-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
MgCl₂
CaCl₂
Histone (Product No. H 4524)
Cold ATP
[γ-³²P] ATP
Phosphatidyserine (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:

<table>
<thead>
<tr>
<th>Vol</th>
<th>Reagent</th>
<th>Final Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 µl</td>
<td>0.5 M HEPES (pH 7.4)</td>
<td>20 mM</td>
</tr>
<tr>
<td>10 µl</td>
<td>100 mM MgCl₂</td>
<td>10 mM</td>
</tr>
<tr>
<td>1 µl</td>
<td>10 mM CaCl₂</td>
<td>0.1 mM</td>
</tr>
<tr>
<td>10 µl</td>
<td>2 mg/ml histone</td>
<td>200 µg/ml</td>
</tr>
<tr>
<td>1 µl</td>
<td>10 mM ATP</td>
<td>100 µM</td>
</tr>
<tr>
<td>10 µl</td>
<td>Lipid Mix (see above)</td>
<td>*</td>
</tr>
<tr>
<td>0.1 µl</td>
<td>[γ³²P]ATP**</td>
<td>trace</td>
</tr>
<tr>
<td>63.9 µl</td>
<td>distilled water</td>
<td>------</td>
</tr>
<tr>
<td>100 µl</td>
<td>= Total volume</td>
<td></td>
</tr>
</tbody>
</table>

*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
- MgCl₂
- PKC Epsilon substrate peptide: (ERMRPRKRQGSVRRRV)
- Cold ATP
- [γ³²P]ATP
- Phosphatidylserine (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.

6. Prepare the reaction mix as follows:

<table>
<thead>
<tr>
<th>Vol.</th>
<th>Reagent</th>
<th>Final Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4 µl</td>
<td>0.5 M HEPES (pH 7.4)</td>
<td>20 mM</td>
</tr>
<tr>
<td>6 µl</td>
<td>100 mM MgCl₂</td>
<td>10 mM</td>
</tr>
<tr>
<td>6 µl</td>
<td>1 mM EGTA</td>
<td>0.1 mM</td>
</tr>
<tr>
<td>6 µl</td>
<td>1 mg/ml substrate peptide</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>0.6 µl</td>
<td>10 mM ATP</td>
<td>100 µM</td>
</tr>
<tr>
<td>6 µl</td>
<td>Lipid Mix (see above)</td>
<td>*</td>
</tr>
<tr>
<td>0.1 µl</td>
<td>[γ-32P]ATP**</td>
<td>trace</td>
</tr>
<tr>
<td>32.9 µl</td>
<td>distilled water</td>
<td>-----</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>PKC Isozyme Reference Guide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoform</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>alpha</td>
</tr>
<tr>
<td>beta I</td>
</tr>
<tr>
<td>beta II</td>
</tr>
<tr>
<td>gamma</td>
</tr>
<tr>
<td>delta</td>
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<td>epsilon</td>
</tr>
<tr>
<td>eta</td>
</tr>
<tr>
<td>theta</td>
</tr>
<tr>
<td>zeta</td>
</tr>
</tbody>
</table>

Activity Calculation:

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

where total cpm in assay = \( X \) cpm (see step 14) \times 100 µl reaction mix/assay ÷ 5 µl reaction mix

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

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