## Certificate of Analysis

# Protein Kinase C:

| Part No. | Size |
|---|---|
| V526A | 1µg (2 × 0.5µg) |

Part# 9PIV526

Revised 11/10

**Description:** Protein Kinase C (PKC) plays a crucial role in the initial events of signal transduction. Normal activation of PKC relies on the stimulation of phospholipid hydrolysis that follows a growth factor:receptor interaction. For example, phospholipase C mediates the hydrolysis of membrane inositol phospholipids to diacylglycerol (DAG) and inositol 1,4,5-trisphosphate, which in turn triggers the release of calcium from intracellular stores. These products activate PKC by mediating its translocation from the cytosol to the membrane. Activated PKC subsequently phosphorylates serine and threonine residues on a variety of intracellular proteins. Many of the PKC target substrates are components of signal transduction pathways and include proteins that regulate ion channels, calcium- and calmodulin-binding proteins, growth factor receptors, structural and regulatory proteins of the cytoskeleton, components of the transcriptional machinery, efflux pumps and many other proteins. For reviews on PKC, see references 1 and 2.

In addition to its central role in normal, regulated signal transduction, PKC is instrumental in the oncogenic process. Tumor-promoting phorbol esters are selective and potent activators of PKC and substitute for DAG, presumably by binding the regulatory domain of the enzyme. In contrast to the transitory activation of PKC during normal signal transduction, the high-affinity, prolonged interaction of phorbol esters with PKC is thought to sustain the mitogenic response during tumorigenesis. See references 3 and 4 for reviews on PKC involvement in tumorigenesis.

Protein Kinase C is an 82kDa monomeric enzyme consisting of a C-terminal catalytic domain and a cysteine-rich N-terminal regulatory domain. PKC is purified from rat brain according to the method of Walton *et al.* (5). The regulatory domain contains the sites for calcium- and phospholipid-binding and a pseudosubstrate, the target for PKC autophosphorylation. There are over ten PKC isozymes known to date, and several of these isozymes demonstrate tissue specificity.

Many of the PKC target substrates are components of signal transduction pathways and include proteins that regulate ion channels, calcium- and calmodulin-binding proteins, growth factor receptors, structural and regulatory proteins of the cytoskeleton, components of the transcriptional machinery, efflux pumps and many other proteins (2,6). Specific PKC substrates include: $Neurogranin_{(28-43)}$ (Cat.# V5611), MARCKS, EGF Receptor (Cat.# V5551), insulin receptor, pp60src, p21 ras, vinculin, talin, myelin basic protein, MAP kinase, topoisomerase I, RNA polymerase II, histones, lamin B, synapsin I, tyrosine hydroxylase, glycogen synthase and other proteins. PKC is activated by calcium and phospholipids. PKC is directly inhibited by calphostin, staurosporine and isoquinoline sulphonamide (H-7 and H-9). PKC is indirectly inhibited by calmodulin antagonists, polymixin B and spermine. Kinase activity is stimulated more than eightfold in the presence of 0.6mg/ml phosphatidylserine.

**Protein Concentration:** 25µg/ml.

**Source:** Purified from rat brain according to the method of Walton *et al* (5). The purified PKC consists primarily of α, β and γ isoforms with lesser amounts of δ and ζ isoforms.

**Storage Buffer:** 20mM Tris-HCl (pH 7.5), 2mM EGTA, 2mM EDTA, 1mM DTT, 10mM potassium phosphate (pH 7.5), 50% glycerol and 0.05% Triton® X-100.

**Storage Conditions:** See the Product Information Label for storage recommendations and expiration date. Avoid multiple freeze-thaw cycles or exposure to frequent temperature changes.

**Unit Definition:** One unit is the amount of kinase needed to transfer 1 nanomole of phosphate per minute at 30°C using Type III-S histone as the substrate and phosphatidylserine at 0.6mg/ml as an activator. See Activity Assay Conditions for buffer and conditions.

## Quality Control Assays

### Activity Assay Conditions

The reaction buffer is 20mM HEPES (pH 7.4), 1.67mM $CaCl_2$, 1mM DTT, 1mg/ml Type III-S histone, 10mM $MgCl_2$ and 0.15mM γ-[$^{32}$P] ATP (0.05mCi/ml); ± 0.6mg/ml phosphatidyl serine. The reaction is run for 2 minutes at 30°C, terminated with phosphoric acid, spotted on Whatman® P81 filters and counted.

**Purity:** The enzyme is greater than 90% pure as judged by SDS-PAGE analysis and Coomassie® blue staining, with a primary band at 82kDa.

**Stimulation:** Kinase activity is stimulated more than eightfold by addition of 0.6mg/ml phosphatidylserine.

**Specific Activity:** The specific activity must be greater than 800 units/mg. See the Product Information Label.

Signed by: J. Stevens

J. Stevens, Quality Assurance


**Promega Corporation**

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# Usage Information

## 1. Assay Protocol

1. Assay PKC activity for 2 minutes at 30°C in a reaction (60µl) containing 20mM HEPES (pH 7.4), 1.0mM DTT, 1mg/ml Type III-S histone (Sigma Cat.# H5505), 10mM $MgCl_2$, 1.7mM $CaCl_2$ and 0.15mM [$\gamma$-$^{32}$P]ATP (1mCi[$\gamma$-$^{32}$P]ATP/reaction) in the presence and absence of 600µg/ml phosphatidylserine.
2. Terminate the reaction with 30µl of 1.5% $H_3PO_4$ and spot 45µl onto Whatman® P81 phosphocellulose filters. Wash filters five times for 5 minutes each in 0.5% $H_3PO_4$. Dry filters and count in a liquid scintillation counter.

## 2. Related Products

| Product | Size | Cat.# |
|---|---|---|
| Neurogranin$_{(28–43)}$ (PKC) Peptide Substrate | 1mg | V5611 |
| PepTag® Non-Radioactive PKC Assay | 120 reactions | V5330 |
| Myristoylated Protein Kinase C Peptide Inhibitor | 1mg | V5691 |
| SignaTECT® Protein Kinase C Assay System | 96 reactions | V7470 |
| Kinase-Glo® Luminescent Kinase Assay | 10ml* | V6711 |
| Kinase-Glo® Plus Luminescent Kinase Assay | 10ml* | V3771 |

*Available in other sizes.

## 3. References


1. Azzi, A., Boscoboinik, D. and Hensey, C. (1992) The protein kinase C family. *Eur. J. Biochem.* **208**, 547–57.
2. Kikkawa, U. and Nishizuka, Y. (1986) The role of protein kinase C in transmembrane signaling. *Ann. Rev. Cell Biol.* **2**, 149–78.
3. Newton, A.C. (1997) Regulation of protein kinase C. *Curr. Opin. Cell. Biol.* **9**, 161–7.
4. Rotenberg, S.A. and Weinstein, I.B. (1991) In: *Biochemical and Molecular Aspects of Selected Cancers,* Vol. 1, Academic Press, Inc., New York.
5. Walton, G.M. *et al.* (1987) A three-step purification procedure for protein kinase C: Characterization of the purified enzyme. *Anal. Biochem.* **161**, 425–37.
6. Jaken, S. (1996) Protein kinase C isozymes and substrates. *Curr. Opin. Cell Biol.* **8**, 168–73.



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