

Certificate of Analysis

Product	PKC peptide substrate, QKRPSQRSKYL
Cat No	PKS-015-01
Lot No	504-240502
Description	The synthetic peptide QKRPSQRSKYL can be used as a substrate for protein kinase C (PKC) in <i>in vitro</i> kinase assays. It is phosphorylated by PKC with a K_m of 7 microM. M.W. 1,391
Purity	85 - 90 % (by HPLC)
Form	Lyophilized powder Reconstitution of 1 mg in 1.4 ml H ₂ O dest. results in a 500 microM solution used in the PKCalpha activity assay.
Package size	1 mg
Storage condition	-20 °C
Shipment conditions	room temperature

References

Yasuda I, Kishimoto A, Tanaka S, Tominaga M, Sakurai, Nishizuka Y (1990) A synthetic peptide substrate for selective assay of protein kinase C. *Biochem Biophys Res Commun* 166:1220-7.

Material for in vitro research use only. Not for pharmaceutical or drug application. Material does not contain any animal products such as albumin.

AVOID FREEZE/THAW CYCLES

PKCalpha *in Vitro* Kinase Assay

Assay Components

One-For-All-Buffer (OFAB): 20 mM Tris-HCl, 25 mM beta-glycerol phosphate, 5 mM EGTA, 1 mM sodium orthovanadate, 1 mM DTT, pH 7.5

Substrate: PKC substrate peptide (QKRPSQRSKYL), 500 microM

Lipid activator: phosphatidylserine, 0.5 mg/ml; diacylglycerol, 0.5 mg/ml diglyceride, sonicated on ice for 1 minute immediately before use

Protein kinase: PKC, 1 - 5 ng / microliter diluted in OFAB, directly before use

Magnesium/ATP Cocktail: 75 mM MgCl₂, 500 microM ATP

Diluted [γ -³²P]ATP: Mix 197 microliter Magnesium/ATP cocktail with 3 microliter (30 microCi) [γ -³²P]ATP (3,000 Ci/mmol, e.g. from Hartmann Analytic, Braunschweig, Germany)

Assay Procedure

All compounds are pipetted into a microcentrifuge tube

1. Add 10 microliter OFAB
2. Add 10 microliter PKC substrate peptide QKRPSQRSKYL, 500 microM
3. Add 10 microliter PKC enzyme (10 - 50 ng/ assay)
4. Add 10 microliter lipid activator, freshly prepared
5. Add 10 microliter of the diluted [γ -³²P]ATP
6. Incubate 10 min at 30 °C.
7. Stop the reaction by setting samples on ice
8. Remove 10 microliter and spot on P81 paper (let bind to the paper for 30 sec)
9. Immerse the paper in 0.75% phosphoric acid, gently shake on a rotator
10. Wash 3 x with phosphoric acid
11. Wash 1 x with acetone
12. Dry under infrared light
13. Read in scintillation counter or Instant Imager