

Certificate of Analysis

Product Cat.No.	PKA peptide substrate, GRTGRRNSI PKS-014-01
Lot No	PT0302060505
Description	The synthetic peptide GRTGRRNSI can be used as a substrate for cAMP dependent protein kinase (PKA) in <i>in vitro</i> kinase assays. It is phosphorylated by PKA with a K_m of 0.1 microM. M.W. 1,016
Purity	90 - 95 % (by HPLC)
Form	Lyophilized powder Reconstitution of 1 mg in 2 ml H ₂ O dest. results in a 500 microM solution used in the PKA activity assay.
Package size	1 mg
Storage condition	-20 °C
Shipment conditions	room temperature

References

Mitchell RD, Glass DB, Wong CW, Angelos KL, Walsh DA (1995) Heat-stable inhibitor protein derived peptide substrate analogs: phosphorylation by cAMP-dependent and cGMP-dependent protein kinases. *Biochemistry* 34:528-34.

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

PKA *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: PKA substrate peptide (GRTGRRNSI), 500 microM

Protein kinase: PKA, 2.5 -10 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 on ice

1. Add 10 microliter OFAB
2. Add 10 microliter PKA substrate peptide, 500 microM
3. Add 10 microliter PKA (25 -100 ng/assay)
4. Add 10 microliter of the diluted [γ -³²P]ATP
5. Incubate 10 min at 30 °C.
6. Stop the reaction by setting samples on ice
7. Remove 10 microliter and spot on P81 paper (let bind to the paper for 30 sec)
8. Immerse the paper in 0.75% phosphoric acid, gently shake on a rotator
9. Wash 3 x with phosphoric acid
10. Wash 1 x with acetone
11. Dry under infrared light
12. Read in scintillation counter or Instant Imager