

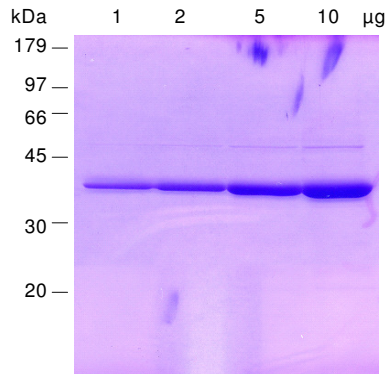
Certificate of Analysis

Product	PKA, catalytic alpha subunit (PRKACA), active human recombinant, expressed in E. coli, N-His-fusion protein	
Cat No	PK-023-01	
Lot No	170304	
Description	Purified human recombinant catalytic subunit alpha of cAMP dependent protein kinase (PKA), expressed in E. coli. Active form. Suitable for labeling PKA substrates. Features a polyhistidine tag to facilitate removal of the enzyme from the reaction mixture. Sequence based calculated M.W. 42,301 Approved HUGO gene symbol: PRKACA Synonyms: protein kinase A, cAMP dependent protein kinase	
Quality	Protein concentration (Bradford with BSA as standard)	1.7 mg/ml
	Purity	> 95 % by SDS PAGE
	Specific activity (* 1 Unit is defined as 1 picomole phosphate transferred to PKA peptide (GRTGRRNSI) per min at 30 °C)	1,600,000 Units*/ mg
	Protease activity (Twining test)	none
Form	Liquid. In 30 mM K-phosphate, 150 mM KCl, 1 mM EDTA, 1 mM DTT, 50 % glycerol, pH 7.4.	
Package size	50 microgram	
Storage condition	-70 °C	
Shipment conditions	dry ice	

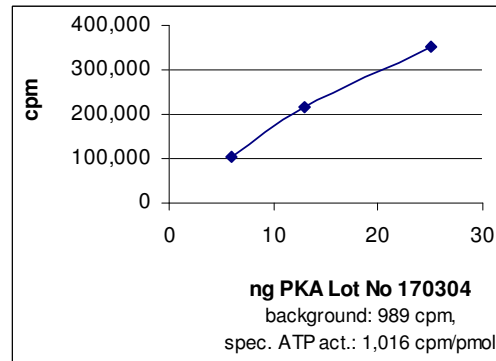
Material for in vitro research use only. Not for pharmaceutical or drug application. Material does not contain any animal products such as albumin.

AVOID FREEZE/THAW CYCLES

SDS-PAGE analysis



Activity determination



Amino acid sequence information

M11 corresponds to M1 of NM_002730

MRGSHHHHHH	GSMGNAAA	KGSEQESVKE	FLAKAKEDFL	KKWESPAQNT	50
AHLDQFERIK	TLGTGSFGRV	MLVKHKETGN	HYAMKILDKQ	KVVKLKQIEH	100
TLNEKRILQA	VNFPFLVKLE	FSFKDNSNLY	MVMEYVPGGE	MFSHLRRIGR	150
FSEPHARFYA	AQIVLTFEYL	HSLDLIYRDL	KPENLLIDQQ	GYIQVTDFFG	200
AKRVKGRWT	LCGTPEYLAP	EIILSKGYNK	AVDWWALGVL	IYEMAAGYPP	250
FFADQPIQIY	EKIVSGKVR	PSHFSSDLKD	LLRNLLQVDL	TKRFGNLKNG	300
VNDIKNHKWF	ATTDWIAIYQ	RKVEAPFIPK	FKGPGDTSNF	DDYEEEEIRV	350
SINEKCGKEF	SEFLEA				366

***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 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 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

Reference

Skalhegg B.S., Tasken K. (2000) *Front. Biosci.* 5, D678-693.