PKA [GST-tagged]
Kinase

Alternate Names: cAMP-dependent protein kinase catalytic subunit alpha isoform 1A kinase alpha, cAMP-dependent protein kinase catalytic subunit alpha EC 2.7.1.37, PKA C alpha, PKACA, Protein kinase A catalytic subunit

Cat. No. **66-0014-050**
Lot. No. **2125**

Quantity: 50 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

Background by Sir Philip Cohen

Protein ubiquitylation and protein phosphorylation are the two major mechanisms that regulate the functions of proteins in eukaryotic cells. However, these different posttranslational modifications do not operate independently of one another, but are frequently interlinked to enable biological processes to be controlled in a more complex and sophisticated manner. Studying how protein phosphorylation events control the ubiquitin system and how ubiquitylation regulates protein phosphorylation has become a focal point of the study of cell regulation and human disease. Cloning of human cyclic AMP-dependent protein kinase A (PKA) was first described by Tasken *et al.* (1996). PKA mediates many of the intracellular actions of the "second messenger" cyclic AMP, and its roles include controlling the rate at which some proteins are degraded. For example, it triggers the degradation of GRIP1 (glucocorticoid receptor-interacting protein 1) by the ubiquitin proteasome system (UPS) (Hoang *et al.*, 2004). On the other hand, PKA-mediated phosphorylation inhibits the degradation of β-catenin by the UPS (Hino *et al.*, 2005), while the PKA-mediated phosphorylation of RGS13 (Regulator of G-protein Signaling 13) prevents degradation by the UPS, leading to enhanced expression of RGS13. Since RGS13 inhibits PKA-induced gene transcription programmes, its phosphorylation represent a negative feedback control

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Physical Characteristics

Species: human

Source: *E. coli*

Quantity: 50 µg

Concentration: 1 mg/ml

Formulation: 50 mM Tris/HCl pH 7.5, 0.1 mM EGTA, 150 mM NaCl, 270 mM sucrose, 0.03% Brij, 0.1% β-Mercaptoethanol, 1 mM Benzamidine, 0.2 mM PMSF

Molecular Weight: ~67.9 kDa

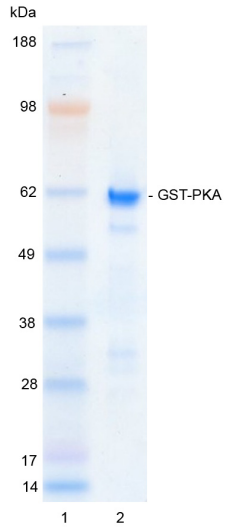
Purity: >85% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg GST-PKA



Protein Identification:
Confirmed by mass spectrometry.

Activity Assay:
The specific activity of GST-PKA was determined using the method described by Hastie *et al.* (2006) with the enzyme being assayed at several concentrations. GST-PKA was incubated for 10 minutes at 30˚C in kinase reaction buffer in the presence of KEMPtide substrate (30 µM) and [γ-^{32}P]ATP (100 µM). Duplicate reactions were stopped by spotting the assay mixture onto Whatman P81 paper – capturing the phosphorylated substrate. The radioactivity incorporated was measured on a scintillation counter and the enzyme's mean specific activity was calculated.

GST-PKA specific activity:
1394.0 Units/mg (1394.0 Units/ml)

1 Unit = 1 nmole of phosphate incorporated into the substrate in 1 minute

Substrate: KEMPtide (LRRASLG)

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Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

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Background by Sir Philip Cohen

Continued from page 1

mechanism to restrict PKA-dependent gene transcription (Xie *et al.*, 2010). It is likely that PKA will be found to regulate the polyubiquitylation and degradation of many other proteins in the future.

References:

Hastie CJ, McLauchlan HJ, Cohen P (2006) Assay of protein kinases using radiolabeled ATP: a protocol. *Nat Protoc* **1**, 968-71.

Hino S, Tanji C, Nakayama KI, Kikuchi A (2005) Phosphorylation of beta-catenin by cyclic AMP-dependent protein kinase stabilizes beta-catenin through inhibition of its ubiquitination. *Mol Cell Biol* **25**, 9063-72.

Hoang T, Fenne IS, Cook C, Borud B, Bakke M, Lien EA, Mellgren G (2004) cAMP-dependent protein kinase regulates ubiquitin-proteasome-mediated degradation and subcellular localization of the nuclear receptor coactivator GRIP1. *J Biol Chem* **279**, 49120-30.

Tasken K, Solberg R, Zhao Y, Hansson V, Jahnsen T, Siciliano MJ (1996) The gene encoding the catalytic subunit C alpha of cAMP-dependent protein kinase (locus PRKACA) localizes to human chromosome region 19p13.1. *Genomics* **36**, 535-8.

Xie Z, Yang Z, Druey KM (2010) Phosphorylation of RGS13 by the cyclic AMP-dependent protein kinase inhibits RGS13 degradation. *J Mol Cell Biol* **2**, 357-65.

Background kindly written by:

Sir Philip Cohen FRS, FRSE
University of Dundee

Director of the Medical Research Council Protein Phosphorylation Unit (1990-2012)

Director of the Scottish Institute for Cell Signalling incorporating the Protein Ubiquitylation Unit (2008-2012)

Co-Director of the Division of Signal Transduction Therapy (1998-2012)

Deputy Director of the Division of Signal Transduction Therapy (from July 2012)

Professor Cohen's research group is studying the interplay between protein phosphorylation and protein ubiquitylation in the regulation of innate immunity.

Physical Characteristics

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Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCPKERAEISM
LEGAVLDIRYGVSRIAYSKDFETLKVDFL
SKLPEMLKMFEDRLCHKTYLNGDHVTHPD
FMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQATF
GGGDHPPKSDLEVLFQGPLGS***G***NAAAAMG
NAAAAKKGSEQESVKEFLAKAKEDFLK
KWESPAQNTAHLDQFERIKTLGTGSFGRVM
LVKHKETGNHYAMKILDKQKVVKLKQIEHTL
NEKRILQAVNFPFLVKLEFSFKDNSNLYM
VMEYVPGGEMFSHLRRIGRFSEPHARFYAAQ
IVLTFEYLHSLDLIYRDLKPENLLIDQQ
GYIQVTDFGFAKRVKGRTWTLCGTPEYLA
PEIILSKGYNKAVDWWALGVLIYEMAAGYP
PFFADQPIQIYEKIVSGKVRFPSHFSS
DLKDLLRNLLQVDLTKRFGNLKNGVNDIKN
HKWFATTDWIAIYQRKVEAPFIPKFKG
PGDTSNFDDYEEEEIRVSINEKCGKEFSEF

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
PKA (regular text): Start ***bold italics*** (amino acid residues 2-351)
Accession number: NP_002721.1

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