

Product: c-AMP dependent Protein Kinase A catalytic subunit α , recombinant
Catalog #: 12-4329
Amount: 10 μ g

DESCRIPTION:

Recombinant c-AMP dependent Protein Kinase A catalytic subunit α (rPKAc-a) cAMP-dependent PKA is an ubiquitous serine/threonine protein kinase present in a variety of tissues (e.g. brain, skeletal muscle, heart). The intracellular cAMP level regulates cellular responses by altering the interaction between the catalytic C and regulatory R subunits of PKA. The inactive tetrameric PKA holoenzyme R2C2 is activated when cAMP binds to R2, which dissociates the tetramer to R2 cAMP 4 and two active catalytic subunits. Free Catalytic subunits of PKA can phosphorylate a wide variety of intracellular target proteins. In response to hormone-induced high cAMP levels, PKA phosphorylates glycogen synthetase (inhibition of the enzyme activity) and phosphorylase kinase to block glycogen synthesis. Different isoforms of catalytic and regulatory subunits suggest specific functions. The recombinant PKA catalytic subunit α is a 41kDa protein. The α -isoform is the predominant form with a broad tissue distribution and can be used for *in vitro* enzymological studies of neural and hormonal signal transduction or to phosphorylate target proteins *in vivo* including Ion channels, transcriptional activator proteins and regulatory enzymes of glycogen metabolism.

SOURCE:

Eschechiria coli

PURITY:

> 95%, as determined by SDS-PAGE

UNIT DEFINITION:

One unit is defined as the amount of recombinant PKA catalytic subunit α , required to incorporate 1nmol of phosphate into the specific substrate peptide kemptide (LRRASIG) in one minute at 30°C.

SPECIFIC ACTIVITY:

The specific activity of the recombinant PKA catalytic subunit α , is >10.000 U/mg. Enzymatic activity >15.000.000 units/mg (based on kemptide phosphorylation).

FORM:

Purified, in 25mM potassium phosphate (pH 6.5), 5mM 2-mercaptoethanol, 5mM EDTA, 150mM NaCl and 50% glycerol.

STORAGE:

-20°C (aliquot), avoid freezing and thawing cycles

ASSAY CONDITIONS:

Roskoski-Assay: Roskoski, R., Jr. (1983) *Methods Enzymol.* 99, 3-6

Protein kinase activity can be measured using a modified radioactive assay according to Roskoski *et al.*: The assay will be performed in a mixture containing 50mM MOPS (pH7.0), 10mM MgCl₂, 0.25 mg/ml bovine serum albumin, 100 IJM Kemptide (peptide substrate), 100 IJM unlabeled ATP mixed with [³²P] ATP (500-1000 cpm/pmol) and Ca subunit in a final volume of 50 IJL. Reaction is started by addition of the Ca subunit and can be stopped after a 5-minutes incubation at 30°C by spotting the reaction mix onto Whatman P-81 filters and soaking the filters four times in 75mM phosphoric acid (10 ml per sample) for at least 5 minutes. After four washing steps rinse filters with ethanol, dry and count.

For the detection of phosphorylation in substrate proteins the phosphotransferase reaction can alternatively be stopped by taking aliquots of the mixture and adding SDS sample buffer. The phosphorylation status of the substrate proteins can subsequently be analysed using SDS PAGE and autoradiography.

Zimmermann, B. (1999) *Journal of Biological Chemistry.*274, 9, 5370-7

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