cAMP-dependent Protein Kinase (PKA), Catalytic Subunit

1-800-632-7799
info@neb.com
www.neb.com

P6000S 0181504 16041

P6000S

100,000 units **2,500,000 U/ml** **Lot: 0181504**

RECOMBINANT **Store at –20°C** **Exp: 4/16**

Description: The catalytic subunit of cAMP-dependent Protein Kinase (PKA) is a serine/threonine protein kinase, which combines, in the absence of cAMP, with the regulatory subunit to form the inactive PKA holoenzyme. Since this is the free catalytic subunit alone, no cAMP is required for activation (1,2).

When purified from mammalian tissue, the PKA catalytic subunit is always phosphorylated at T197, essential for catalysis. Most likely a heterologous kinase is responsible for this *in vivo* phosphorylation of PKA. Although the fully active PKA expressed in *E. coli* autophosphorylates on both T197 and S338, this does not reflect the mechanism used in eukaryotic cells (3).

New Reaction Buffer

Recognition Determinants: The recognition motif for phosphorylation by PKA is RRX**S/T**Y, where Y tends to be a hydrophobic residue. A Phe in the nearby sequence tends to be a negative determinant for phosphorylation by PKA. Some variations with regard to spacing and basic residues are permissible (2,4).

Source: Isolated from a strain of *E. coli* that carries a clone of the murine PKA catalytic subunit (α isoform) under control of a T7 expression system (1,2) (cDNA kindly provided by Dr. G.S. McKnight).

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C), 1 mM Na_2EDTA, 2 mM DTT and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer for Protein Kinases

Reaction Conditions: 1X NEBuffer for Protein Kinases, supplemented with 200 µM ATP and gamma-labeled ATP to a final specific activity of 100–500 µCi/µmol. **Incubate at 30°C.**

1X NEBuffer Protein Kinases:
50 mM Tris-HCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 2 mM DTT, 0.01% Brij 35, pH 7.5 @ 25°C

Note that optimal incubation times and enzyme concentrations must be determined empirically for each particular substrate.

Unit Definition: One unit is defined as the amount of PKA catalytic subunit required to catalyze the transfer of 1 pmol of phosphate to Kemptide, LRRA**S**LG (100 µM, NEB #P6001) in 1 minute at 30°C in a total reaction volume of 25 µl.

Specific Activity: ~ 5,000,000 units/mg

Molecular Weight: 38 kDa

Quality Assurance: PKA contains no detectable protease or phosphatase activities.

Quality Control Assays

Protease Activity: After incubation of 20,000 units of cAMP-dependent Protein Kinase (PKA), catalytic subunit with a standard mixture of proteins for 2 hours at 30°C, no proteolytic activity could be detected by SDS-PAGE analysis.

Phosphatase Activity: After incubation of 20,000 units of cAMP-dependent Protein Kinase (PKA), catalytic subunit with 50 mM *p*-nitrophenyl phosphate for 2 hours at 30°C, no phosphatase activity could be detected by spectrophotometric analysis.

Heat Inactivation: 65°C for 20 minutes.

References:

1. Uhler, M.D., et al. (1986) *Proc. Natl. Acad. Sci. USA* 83,1300–1304.
2. Slice, L.W. and Taylor, S.S. (1989) *J. Biol. Chem.* 264, 20940–20946.
3. Moore, M.J. et al. (2002) *J. Biol. Chem.* 277, 47878–47884.
4. Zetterqvist, O.Z. et al. (1990) in *Peptides and Protein Phosphorylation* (B.E. Kemp, ed), pp. 171–187, CRC Press, Inc., Boca Raton.

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CERTIFICATE OF ANALYSIS

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