**cAMP-dependent Protein Kinase (PKA), catalytic subunit**

Both T197 and S338, this does not reflect the mechanism used in eukaryotic cells (3).

**Recognition Determinants:** The recognition motif for phosphorylation by PKA is RRXk/TY, where k 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 Na2EDTA, 2 mM DTT and 50% glycerol.

**Reagents Supplied with Enzyme:**
- 10X PKA Reaction Buffer

**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.

**References:**

**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:**

**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 PKA 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).

**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).

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