Ca2+/Calmodulin-Dependent Protein Kinase II (CaMKII)

**Recognition Determinants:** The minimal recognition motif for phosphorylation by CaMKII is RXxs/T. A more recent report suggests the presence of positive determinants at the -5, -2 and +1 positions in addition to the -3R. Thus, CaMKII preferentially phosphorylates substrates with motifs: HydXRXXS/T and HydXRNBS/X/T, respectively, where Hyd represents a hydrophobic, X any, and NB a non-basic amino acid residue (3).

**Source:** Isolated from *Spodoptera frugiperda* (Sf9) cells infected with recombinant baculovirus carrying the truncated rat CaMKII (kindly provided by Dr. H. Shulman).

**Reagents Supplied with Enzyme:**
- 10X NEBuffer for Protein Kinases (PK)
- 1X CaCl2 (20 mM)
- 10 mM MgCl2
- 100 µM ATP (NEB #P0756), 1.2 µM calmodulin and 2 mM CaCl2
- Incubate for 10 minutes at 30°C. The dilution of CaMKII should not exceed 20,000–50,000 units/ml to ensure the suggested rate of autophosphorylation.

**Phosphorylation with CaMKII:**
1. **CaMKII Activation:** Dilute the desired amount of CaMKII in 1X NEBuffer for PK (NEB #B6022) supplemented with 200 µM ATP (NEB #P0756), 1.2 µM calmodulin and 2 mM CaCl2. Incubate for 10 minutes at 30°C. The ATP concentration should be about 100 µM.
2. **Substrate Phosphorylation:** Mix the substrate with 1X NEBuffer for PK supplemented with ATP. Add the activated CaMKII. Incubate at 30°C.

**Usage Note:** The ATP concentration should be at or near saturation (5–10-fold over Km). Apparent Km values of ATP for most protein kinases are below 100 µM.

However, if the objective is to measure enzyme activity using gamma-labeled ATP, it is best to use 100–200 µM ATP in order to have higher specific activity of gamma-labeled ATP (100–500 cpm/pmole). Also, an excess of substrate should be used, and the level of phosphorylation should not exceed 10% for determination of the initial rate.

1. **NEBuffer for PK:**
   - 50 mM Tris-HCl
   - 10 mM MgCl2
   - 0.1 mM EDTA
   - 1 mM DTT
   - pH 7.5 @ 25°C

5,000 units 500,000 U/ml Lot: 0151401
RECOMBINANT Store at –70°C Exp: 1/15

**Description:** Ca2+/Calmodulin-Dependent Protein Kinase II (CaMKII) is a serine/threonine kinase. It is a Ca2+/calmodulin-dependent, truncated monomer (1–325 amino acid residues) of the α subunit. Autophosphorylation of threonine 286 in the presence of Ca2+ and calmodulin activates CaMKII and produces substantial Ca2+/calmodulin-independent activity (1,2).

**Reaction Conditions:** Prior to substrate phosphorylation, CaMKII should be activated by autophosphorylation with ATP/Mg2+ in the presence of CaCl2 and calmodulin. Neither CaCl2 nor calmodulin are required for the subsequent phosphorylation of exogenous substrate.

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

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To phosphorylate a protein or peptide substrate to completion, the ATP concentration should be about 5-fold over the limited substrate concentration. Higher enzyme concentration and prolonged incubation times should be employed (4).

1. **NEBuffer for PK:**
   - 50 mM Tris-HCl
   - 10 mM MgCl2
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   - 2 mM DTT
   - pH 7.5 @ 25°C

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Unit Definition: One unit is defined as the amount of activated CaMKII required to catalyze the transfer of 1 pmol of phosphate to Autocamtide-2 (CaMKII Peptide Substrate), KKLRRQETVDAL (50 µM), in 1 minute at 30°C in a total reaction volume of 30 µl (5).

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

Molecular Weight: 36 kDa. The apparent molecular weight of CaMKII on SDS-PAGE is about 33 kDa.

Purity: CaMKII has been purified to > 95% homogeneity as determined by SDS-PAGE and Coomassie Blue staining.

Heat Inactivation: 65°C for 20 minutes.

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

Quality Control Assays
Protease Activity: After incubation of 5,000 units of Ca²⁺/Calmodulin-Dependent Protein Kinase II (CaMKII) 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 5,000 units of Ca²⁺/Calmodulin-Dependent Protein Kinase II (CaMKII) with 50 mM p-nitrophenyl phosphate for 2 hours at 30°C, no phosphatase activity could be detected by spectrophotometric analysis.

Notes On Use: Avoid freeze/thaw cycles. Can be stored for 2 weeks or less at –20°C.

References: