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

**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).

**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 HydXRNBXS/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).

**Supplied in:** 100 mM NaCl, 50 mM HEPES (pH 7.5 @ 25°C), 0.1 mM EDTA, 1 mM DTT, 0.01% Brij 35 and 50% glycerol.

**Reagents Supplied with Enzyme:**
- 10X NEBuffer for Protein Kinases (PK)
- 10X CaCl₂ (20 mM)
- 10X Calmodulin (12 μM)
- ATP (10 mM)

**Usage Note:**
- 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/pmol). Also, an excess of substrate should be used, and the level of phosphorylation should not exceed 10% for determination of the initial rate.
- 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).

**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.

**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 dilution of CaMKII should not exceed 20,000–50,000 units/ml to ensure the suggested rate of autophosphorylation.

2. **Substrate Phosphorylation:** Mix the substrate with 1X NEBuffer for PK supplemented with ATP. Add the activated CaMKII. Incubate at 30°C.

**Usage Note:**
- 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/pmol). Also, an excess of substrate should be used, and the level of phosphorylation should not exceed 10% for determination of the initial rate.
- 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).

**1X NEBuffer for PK:**
- 50 mM Tris-HCl
- 10 mM MgCl₂
- 0.1 mM EDTA
- 2 mM DTT
- 0.01% Brij 35
- pH 7.5 @ 25°C

**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 dilution of CaMKII should not exceed 20,000–50,000 units/ml to ensure the suggested rate of autophosphorylation.

2. **Substrate Phosphorylation:** Mix the substrate with 1X NEBuffer for PK supplemented with ATP. Add the activated CaMKII. Incubate at 30°C.

**Usage Note:**
- 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/pmol). Also, an excess of substrate should be used, and the level of phosphorylation should not exceed 10% for determination of the initial rate.
- 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).

**1X NEBuffer for PK:**
- 50 mM Tris-HCl
- 10 mM MgCl₂
- 0.1 mM EDTA
- 2 mM DTT
- 0.01% Brij 35
- pH 7.5 @ 25°C
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), KKLARQETVDAL (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: