

CaMKII- α Kinase

✓ 5 μ g

Orders ■ 877-616-CELL (2355)
orders@cellsignaling.com
Support ■ 877-678-TECH (8324)
info@cellsignaling.com
Web ■ www.cellsignaling.com

rev. 07/23/08

This product is for *in vitro* research use only and is not intended for use in humans or animals.

Description: Purified recombinant full length human CaMKII- α kinase, supplied as a GST fusion protein.

Background: CaMKII is an important member of the calcium/calmodulin-activated protein kinase family, functioning in neural synaptic stimulation and T-cell receptor signaling (1,2). CaMKII has catalytic and regulatory domains. The binding of Ca²⁺/calmodulin to its regulatory domain releases its autoinhibitory effect and activates the kinase (3). The activated CaMKII further autophosphorylates at Thr286 to render the kinase constitutively active (3). The threonine phosphorylation state of CaMKII can be regulated through PP1/PKA. PP1 (protein phosphatase 1) dephosphorylates phospho-CaMKII at Thr286. PKA (protein kinase A) prevents this dephosphorylation through its inhibitory effect on PP1 (4).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system using sf9 cells and a recombinant virus encoding full-length human CaMK2 α (Met1-His478) (GenBank Accession No. NM_171825) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using GSH-agarose.

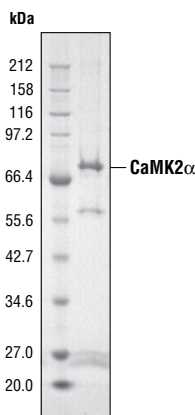


Figure 1. The purity of the GST-CaMKII- α fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Quality Control: The theoretical molecular weight of the GST- CaMK2 α fusion protein is 74 kDa. The purity of the kinase was assessed using SDS-PAGE followed by Coomassie stain [Fig.1]. CaMK2 α kinase activity was determined using a radiometric assay [Fig.2].

Background References:

- (1) Hughes, K. et al. (2001) *J. Biol. Chem.* 276, 36008–36013.
- (2) Barria, A. et al. (1997) *Science* 276, 2042–2045.
- (3) Means, A.R. (2000) *Mol. Endocrinol.* 14, 4–12.
- (4) Makhinson, M. et al. (1999) *J. Neurosci.* 19, 2500–2510.

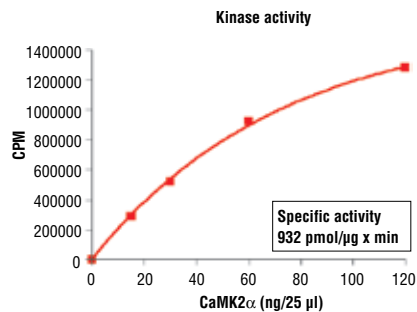


Figure 2. CaMKII- α kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β -glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM MgCl₂, 0.5 mM CaCl₂, 0.03 mg/ml calmodulin, 0.05 mM DTT, 50 μ M ATP, Substrate: Autocamtide (KKALRRQETVDAL) 300 ng/ μ L, and recombinant CaMKII- α : variable.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione. Store at -80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Serine/Threonine Kinase Substrate Screening Kit #7400

Protocol for CaMKII- α Kinase Assay

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

1. Kinase Buffer (10X)

50 mM MOPS, pH 7.2
25 mM β -glycerophosphate
10 mM EGTA
4 mM EDTA
50 mM $MgCl_2$
0.5 mM DTT

2. ATP (10 mM) #9804

3. ^{32}P - γ ATP

4. Autocamtide (1 μ g/ μ l)

5. 5 mM $CaCl_2$ solution containing 0.3 mg/ml Calmodulin

B Suggested Protocol

1. Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 μ M ATP.
2. Dilute [^{32}P] ATP to 0.16 μ Ci/ μ l [^{32}P] ATP with 250 μ M ATP solution.
3. Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
4. Dilute CaMK2 α protein to 12 ng/ μ l with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 μ l diluted CaMKII- α kinase solution, 7.5 μ l Autocamtide (1 μ g/ μ l), 2.5 μ l of 5 mM $CaCl_2$ solution containing 0.3 μ g/ μ l Calmodulin, and 5 μ l 0.16 μ Ci/ μ l [^{32}P] ATP solution.

Final Assay Conditions

5 mM MOPS, pH 7.2
2.5 mM β -glycerophosphate
1 mM EGTA
4 mM $MgCl_2$
0.5 mM $CaCl_2$
0.03 mg/ml Calmodulin
0.05 mM DTT
300 ng/ μ L Autocamtide

6. After 15 minutes terminate reaction by spotting 20 μ l of the reaction mixture onto phosphocellulose P81 paper.
7. Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
8. Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
9. Count samples in a scintillation counter.

Cell Signaling Technology offers a full line of protein kinases, substrates, and antibody detection reagents for high throughput screening. Please direct all inquiries to: drugdiscovery@cellsignal.com.