

CaMKI- γ Kinase

✓ 5 μ g



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Orders ■ 877-616-CELL (2355)
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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 CaMKI- γ (Met1-Met476) kinase, supplied as a GST fusion protein.

Background: The Ca²⁺/calmodulin-dependent kinase (CaMK) family, which is activated in response to elevation of intracellular Ca²⁺, includes CaMKI, CaMKII, CaMKIV and CaMK-kinases (CaMKKs) (1,2). CaMKI is a downstream substrate of CaMKK and has 4 isoforms: CaMKI- α , CaMKI- β , CaMKI- δ and CaMKI- γ . CaMKI is present in most cell types and may be involved in cellular functions including transcription, cytoskeletal organization, axonal growth cone motility and long-term potentiation in neurons (3,4,5,6). CaMKII is also ubiquitously expressed in most cell types. While muscular CaMKII has been linked to activation of mitochondrial biogenesis in muscle hypertrophy response, neuronal CaMKII regulates important neuronal functions, including neurotransmitter synthesis, neurotransmitter release, modulation of ion channel activity, cellular transport, cell morphology and neurite extension, synaptic plasticity, learning and memory and gene expression (7). Like CaMKI, CaMKIV is also a substrate of CaMKKs and is primarily restricted to the nucleus of neurons. CaMKIV regulates gene transcription in neurons through phosphorylation of transcription factors such as CREB and is required for fear memory (8).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full length human CaMKI- γ (Met1-Met476) (GenBank Accession No. NM_020439) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

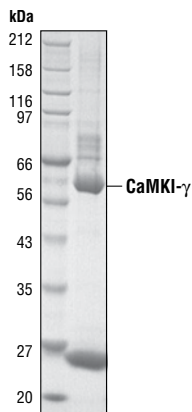


Figure 1. The purity of the GST-CaMKI- γ fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Quality Control: The theoretical molecular weight of the GST-CaMKI- γ fusion protein is 79 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. CaMKI- γ kinase activity was determined using a radiometric assay [Fig.2].

Background References:

- (1) Chin, E.R. (2004) *Proc. Nutr. Soc.* 63, 279–286.
- (2) Mizuno, K. and Giese, K.P. (2005) *J. Pharmacol. Sci.* 98, 191–197.
- (3) Wayman, G.A. et al. (2004) *J. Neurosci.* 24, 3786–3794.
- (4) Gardner, H.P. et al. (2000) *Genomics* 63, 279–288.
- (5) Verploegen, S. et al. (2005) *Blood* 106, 1076–1083.
- (6) Takemoto-Kimura, S. et al. (2003) *J. Biol. Chem.* 278, 18597–18605.
- (7) Yamauchi, T. (2005) *Biol. Pharm. Bull.* 28, 1342–1354.
- (8) Wei, F. et al. (2002) *Nat. Neurosci.* 5, 573–579.

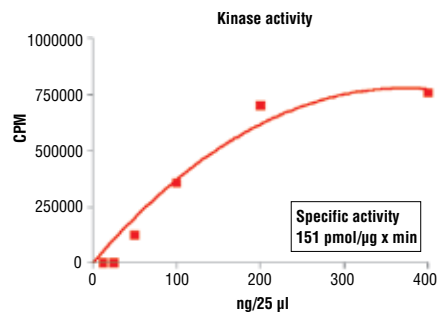


Figure 2. CaMKI- γ kinase activity was measured in a radiometric assay using the following reaction conditions: 4 mM MOPS, pH 7.2, 2.5 mM β -glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 3 mM MgCl₂, 30 ng/ μ l calmodulin, 0.5 mM CaCl₂, 0.05 mM DTT, 50 μ M ATP. Substrate: Autocamtide 2, 300 ng/ μ l and recombinant CaMKI- γ : 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.

Protocol for CaMKI- γ 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)

40 mM MOPS, pH 7.2
25 mM β -glycerophosphate
10 mM EGTA
4 mM EDTA
300 mM $MgCl_2$
0.5 mM DTT
400 ng/ μ L BSA

2. ATP (10 mM) #9804

3. ^{32}P - γ ATP

4. Calmodulin (0.3 mg/ml in 5 mM $CaCl_2$)

5. Autocamtide (KKALRRQETVDAL) 1 μ g/ μ l

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 CaMKI- γ protein (100 ng/ μ l concentration) to 50 ng/ μ l with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 μ l diluted CaMKI- γ kinase solution, 7.5 μ l Autocamtide 2 (1 μ g/ μ l), 2.5 μ l Calmodulin (0.3 mg/ml in 5 mM $CaCl_2$) and 5 μ l 0.16 μ Ci/ μ l [^{32}P] ATP solution.

Final Assay Conditions

- 4 mM MOPS, pH 7.2
2.5 mM β -glycerophosphate
1 mM EGTA
0.4 mM EDTA
30 mM $MgCl_2$
0.05 mM DTT
40 ng/ μ l BSA
30 ng/ μ l calmodulin
0.5 mM $CaCl_2$
300 ng/ μ l autocamtide 2
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.

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