CAMKK2 (Human), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SPC18

Lot No. B281-2
5 µg 0.1 µg/µl

Background:

CAMKK2 (CAMKKb) is a member of the CAMKK family. It is broadly distributed in tissues with highest levels in brain, thymus, spleen, and testis (1). CAMKK2 undergoes intramolecular autophosphorylation, is regulated by Ca2+/calmodulin and phosphorylates CAMKI and CAMKIV on Thr177 and Thr200, respectively. CAMKK2 activates both CAMKI and CAMKIV when coexpressed in Jurkat T cells. CAMKK2 also phosphorylates and regulates the activity of AMPK, which is an important regulator of cellular metabolism in response to metabolic stress (2).

Product Description:

Recombinant full-length human CAMKK2 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_172226.

Gene Aliases:

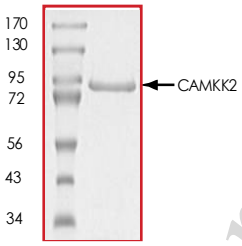
CAMKKb, KIAA0787, MGC15254

Formulation:

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

Purity & Molecular Weight:

The purity was determined to be >90% by densitometry. Approx. MW 88kDa.



Storage:

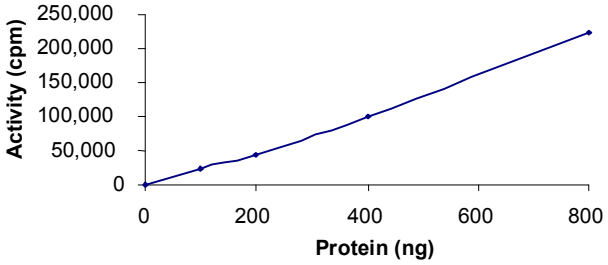
Store product at –70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

1 year at –70 °C from date of shipment.

Specific Activity:

The specific activity was determined to be 11 nmol /min/mg as per Activity Assay Protocol.



Activity Assay Protocol:

Assay activity of the kinase in a 25 µL reaction consisting of 5 µL of 5 X Kinase Assay Buffer, 10 µL of 1 mg/ml the Substrate Solution, 5 µL of diluted kinase and 5 µL of 250 µM ATP solution containing [gamma ^{32}P] ATP (0.167 µCi/µL). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20 µL of the reaction mixture onto phosphocellulose P81 paper. Air-dry the P81 paper and sequentially wash 4 times for approximately 10 minutes each in 1% phosphoric acid with constant gentle stirring. Count the P81 paper in a liquid scintillation counter.

Substrate Solution:

Myelin basic protein (MBP) diluted in distilled H_2O to a final concentration of 1mg/ml.

5 X Kinase Assay Buffer:

25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 20mM $MgC1_2$, 12.5mM MnC12, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

References:

1.Anderson, K.A. et al: Components of a calmodulin-dependent protein kinase cascade. Molecular cloning, functional characterization and cellular localization of Ca2+/calmodulin-dependent protein kinase kinase beta. J Biol Chem. 1998 Nov 27;273(48):31880-9.

2.Hurley, R.L. et al: The Ca2+/calmodulin-dependent protein kinase kinases are AMP-activated protein kinase kinases. J Biol Chem. 2005 Aug 12;280(32):29060-6.

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