



CaMKK beta Positive Control (Full length)

Product Data Sheet

For Research Use Only, Not for use in diagnostic procedures

CaMKK beta Positive Control (Full length)
(Human, full length, recombinant protein expressed *E. coli.*)
Cat# CY-E1185-2

Lot No.
For 200 Assays
(30 units/ μ L x 350 μ L)

Product Description:

Human CaMKK beta full length, residues 1-588, containing a *N*-terminal GST tag, expressed in *E. coli.*, purified by GSH agarose chromatography. The CaMKK beta Positive Control is designed to use for CycLex CaMKK beta Kinase Assay Kit (Cat# CY-1185). The CaMKK beta Positive Control should be added to the well at 50 units/well. For instance, diluted the Positive Control 1:6, use 10 μ L for 1 assay. Unused CaMKK beta Positive Control should be stored at -70°C .

Product Size: Recombinant CaMKK beta: 10,500 units/700 μ L

Formulation: The CaMKK beta Positive Control is supplied frozen in a buffer containing 20mM Hepes-KOH (pH 7.5), 1 % BSA, 1mM EDTA, 1 mM DTT, 50mM NaCl, 0.03 % Brij35 and 50% glycerol.

Source: Human full length CaMKK beta, containing *N*-terminal GST tag, expressed in *E. coli.*

Molecular Weight: The CaMKK beta Positive Control demonstrates a single 90 kDa band by SDS-PAGE analysis.

Purity: GST-CaMKK beta is greater than 85 % pure as determined by SDS-PAGE analysis.

Substrates: CaMKK beta phosphorylates AMPK alpha.

Inhibitors: STO609 is known as selective CaMKK beta inhibitor.

Unit Definition: One unit is defined as the amount of kinase required to incorporate 1 pmol of phosphate into the AMPK alpha 1 per minute at 30°C .

Assay Conditions: Assay CaMKK beta Positive Control in a 50 μ L reaction containing 20 mM Hepes KOH (pH 7.5), 0.1 mM EGTA, 10 mM MgCl_2 , 200 ng/mL Calmodulin, 2 mM CaCl_2 , 5 mM DTT, 100 μ M [γ - ^{32}P] ATP (1 μ Ci), and 250 ng of GST-AMPK alpha 1(1-394). Start the reaction by adding 10 μ L of the Positive Control, diluted 50-fold in a buffer containing 20 mM Hepes KOH (pH 7.5), 0.1 mM EGTA, 10 mM MgCl_2 , 0.03 % Brij35. Incubate for 30 minutes at 30°C . Terminate the reaction by spotting to phosphocellulose P81 paper, and then wash 4 times with 75 mM H_3PO_4 for 15 min and rinse P81 paper with ethanol. Dry filters and count in a liquid scintillation counter.



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Storage and Stability: Stable for 12 months at -70°C from date of shipment. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap. Aliquot the Positive Control to avoid repeated freezing and thawing.

References:

- (1) Hawley SA, Pan DA, Mustard KJ, Ross L, Bain J, Edelman AM, Frenguelli BG, Hardie DG. Cell Metab. 2005; 2(1): 9-19
- (2) Shaw RJ, Kosmatka M, Bardeesy N, Hurley RL, Witters LA, DePinho RA, Cantley LC. PNAS USA. 2004; 101(10): 3329–3335.
- (3) Hawley S, Boudeau J, Reid J, Mustard K, Udd L, Makela T, Alessi D, Hardie DG. J Biol. 2003; 2(4): 28.
- (4) Hurley RL, Anderson KA, Franzone JM, Kemp BE, Means AR, Witters LA. JBC. 2005; 280(32): 29060–29066.
- (5) Hawley SA, Pan DA, Mustard KJ, Ross L, Bain J, Edelman AM, Frenguelli BG, Hardie DG. Cell Metab. 2005; 2(1): 9–19.
- (6) Woods A, Dickerson K, Heath R, Hong S-P, Momcilovic M, Johnstone SR, Carlson M, Carling D. Cell Metab. 2005; 2(1): 21-33.
- (7) Anderson KA, Means RL, Huang Q-H, Kemp BE, Goldstein EG, Selbert MA, Edelman AM, Fremeau RT, Means AR. JBC 1998; 273(48): 31880–31889.

Related Products:

- * CycLex CaMKK beta Kinase Assay Kit: Cat# CY-1185
- * CaMKK beta Positive Control (Full length): Cat# CY-E1185-2

PRODUCED BY

CycLex Co., Ltd.
1063-103 Terasawaoka
Ina, Nagano 396-0002
Japan
Fax: +81-265-76-7618
e-mail: info@cyclex.co.jp
URL: <http://www.cyclex.co.jp>

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