

CaM kinase II Positive Control Product Data Sheet



For Research Use Only, Not for use in diagnostic procedures

CaM kinase II Positive Control

(Human, a.a.1-328, recombinant protein expressed in Sf9 cells) Cat# CY-E1173

> Lot No. For 200 Assays 3 units (30 m units/μL)

Supplied with 100X Calmodulin (25 µg/mL x 0.2 mL).

Product Description: Human Ca²⁺/calmodulin-dependent protein kinase II (CaM kinase II), containing an N-terminal GST tag and a C-terminal His tag, is expressed in recombinant baculovirus infected Sf9 cells and purified by sequentially using GSH agarose and Ni-NTA agarose chromatography. The CaM kinase II Positive Control is designed to use for CycLex CaM kinase II kinase Assay Kit (Cat# CY-1173). The CaM kinase II Positive Control should be added to the well at 15 m units/well. Unused CaM kinase II Positive Control should be stored at -70°C.

Product Size: 3 units/100 μL

Formulation: Supplied frozen in a buffer containing 20 mM Hepes-KOH (pH 7.5), 1 % BSA, 1 mM EDTA, 1 mM DTT, 50 mM NaCl, 0.03 % Brij35 and 50 % glycerol.

Source: Human Ca²⁺/calmodulin-dependent protein kinase II, 1-328, containing an N-terminal GST tag and a C-terminal His tag, expressed in Sf9 cells.

Molecular Weight: 62 kDa doublet bands by SDS-PAGE analysis.

Purity: > 90 % pure as determined by SDS-PAGE analysis.

Substrates: CaM kinase II phosphorylates a number of substrates, including synthetic peptides.

Inhibitors: Selective CaM kinase II inhibitor has not been discovered yet.

Unit Definition: One unit is defined as the amount of kinase required to incorporate 1nmol of phosphate into the syntide-2 per minute at 30°C.

Assay Conditions: Assay activity of CaM kinase II in a 50 μ L reaction containing 20 mM Hepes KOH (pH 7.5), 0.1 mM EGTA, 5 mM MgCl₂, 200 ng calmodulin, 2 mM CaCl₂, 1 mM DTT, 100 μ M [gamma ³²P] ATP (1 μ Ci), and 4 μ g of syntide-2. Start the reaction by adding 10 μ L of the enzyme, diluted 5-fold in a buffer containing 20 mM Hepes KOH (pH 7.5), 1 mM DTT, 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 H₃PO₄ for 15 min and rinse P81 paper with ethanol. Dry filters and count in a liquid scintillation counter.

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 enzyme to avoid repeated freezing and thawing.

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References:

- 1. Andy HUDMON1 and Howard SCHULMAN1 Biochem. J. 364, 593-611, 2002
- Van Eldik, L., and Watterson, M. Calmodulin and Signal Transduction, Academic Press, New York, 1998
- 3. Erondu, N. E. and Kennedy, M. B. Regional distribution of type II Ca2+/calmodulin-dependent protein kinase in rat brain. *J. Neurosci.* 5, 3270-3277, 1985
- 4. Soderling, T. R. Biochim. Biophys. Acta 1297, 131-138, 1996
- 5 Kolodziej, S. J., Hudmon, A., Waxham, M. N., and Stoops, J. K. J. Biol. Chem. 275, 14354–14359, 2000
- 6. Kanaseki, T., Ikeuchi, Y., Sugiura, H., and Yamauchi, T. J. Cell Biol. 115, 1049-1060, 1991
- 7. Brocke, L., Chiang, L. W., Wagner, P. D., and Schulman, H. *J. Biol. Chem.* 274, 22713–22722, 1999

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