



PDK1 Positive Control

Product Data Sheet

For Research Use Only, Not for use in diagnostic procedures

PDK1 Positive Control

(Human, full length, recombinant enzyme expressed in Sf9)

Cat# CY-E1180

Lot No.

For 200 Assays

(40 mUnits / $\mu\text{L} \times 100 \mu\text{L}$)

Product Description: Human full length PDK1, containing a C-terminal His tag, expressed in *Sf9*. Purified by sequentially using phospho-cellulose and Ni-NTA agarose chromatography. The PDK1 Positive control is designed to use for PDK1 assay/Inhibitor Screening Kit [Cat# CY-1180]. The PDK1 Positive Control should be added to the well at 20 m units/well. For instance, diluted positive control 1:20, use 10 μL for 1 assay. Unused PDK1 Positive control should be stored at -70°C .

Product Size: Full length PDK1: 4 units/100 μL

Formulation: The PDK1 Positive Control is supplied frozen in a buffer containing 20mM HEPES-KOH (pH 7.5), 1 % BSA, 1 mM DTT, 50mM NaCl, 0.03 % Brij35 and 50% glycerol.

Source: Human full length PDK1, containing a C-terminal His tag, expressed in *Sf9*.

Molecular Weight: PDK1 demonstrates a single 65 kDa band by SDS-PAGE analysis.

Purity: PDK1 is greater than 85% pure as determined by SDS-PAGE analysis.

Substrates: PDK1 phosphorylates threonine in active loop of many AGC kinases including AKT1 at Thr308

Inhibitors: Specific PDK1 inhibitor has not been discovered yet. However OSU03012 is known as an inhibitor for PDK1.

Unit Definitions: One unit is defined as the amount of kinase required to incorporate 1 nmol of phosphate into the AKT-T308 peptide, per minute at 30°C .

Assay Conditions: Assay activity of PDK1 in a 50 μL reaction containing 20 mM Hepes KOH (pH 7.5), 5 mM MgCl_2 , 1 mM DTT, 100 μM [γ - ^{32}P] ATP (1 μCi), and 4 μg of AKT-T308 peptide. Start the reaction by adding 10 μL of the enzyme, diluted 50-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 . The reaction is terminated by "spotting" a sample onto a phosphocellulose P81 filter paper disc, followed by washing extensively with 70 mM phosphoric acid to remove unincorporated radiolabel. Subsequently rinse the filters with ethanol and dry. The incorporated radioactivity on P81 filter is counted 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.

Related Products:

* PDK1 Assay/Inhibitor Screening Kit: Cat# CY-1180



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General References:

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