AKT1 Positive Control

(Human, a.a.146-480, recombinant protein expressed in Sf9 cells)
Cat# CY-E1168-1

Lot No.
For 200 Assays
5 units (50 m units/µL)

Supplied with 10X BSA (100 µg/mL x 0.25 mL) to make Enzyme Dilution Buffer.

Product Description: Constitutive active form of human AKT1, in which there is one mutation S473D, containing an N-terminal GST tag and a C-terminal His tag, is produced by co-infection with PDK-1, expressing recombinant baculovirus into Sf9 cells, and purified by using GSH agarose chromatography. The AKT1 Positive Control is designed to use for CycLex AKT/PKB Kinase Assay/Inhibitor Screening Kit (Cat# CY-1168). The AKT1 Positive Control should be added to the well at 25 m units/well. Unused AKT1 Positive Control should be stored at -70°C.

Product Size: 5 units/100 µL

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

Dilution: The AKT1 Positive Control should be diluted with Enzyme Dilution Buffer* to avoid inactivating the enzyme activity in low protein concentration condition.

* Enzyme Dilution Buffer: Mix 9-parts of Kinase Buffer of CycLex AKT/PKB Kinase Assay/Inhibitor Screening Kit (Cat# CY-1168) and 1-part of 10X BSA (100 µg/mL)

Source: Human AKT1, 146-480, S473D, containing an N-terminal GST tag and a C-terminal His tag, expressed in Sf9 cells.

Molecular Weight: 92 kDa doublet bands by SDS-PAGE analysis.

Purity: > 85% pure as determined by SDS-PAGE analysis.

Substrates: AKT1 phosphorylates a number of substrates, including GSK-3alpha, Apaf-1, hTERT, Bad and MBP.

Inhibitors: AKT1 specific 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 GST-Bad per minute at 30°C.

Assay Conditions: Assay activity of AKT1 in a 50 µL reaction containing 20 mM Hepes KOH (pH 7.5), 5 mM $MgCl_2$, 1 mM DTT, 100 µM [gamma ^{32}P] ATP (1 µCi), and 4 µg of GST-Bad fusion protein. Start the reaction by adding 10µL 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. Terminate the reaction by adding 600 µL of cold 10 % TCA solution containing 0.2 % Sodium pyrophosphate and stand on ice for 15 min. Filtrate acid insoluble material through GFC filters (Whatman Inc.), wash 4

times with 1 % TCA and rinse filters 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.

References:

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