AKT1 Positive Control

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

Lot No.
For 200 Assays
(50 mUnits / µL x 100 µL)

10X BSA (100 µg/mL x 0.25 mL) is supplied to make an enzyme dilution buffer.

Product Description:
Constitutive active form of human AKT1 in which there is one mutation S473D, containing a *N*-terminal GST tag and a C-terminal His tag was produced by co-infection of PDK-1 and AKT1 (residues 146-480) expressing recombinant baculovirus into sf9 cells. Purified by using GSH agarose chromatography. The AKT1 Positive control is designed to use for CycLex AKT Assay/Inhibitor Screening Kit (Cat# CY-1168). The AKT1 Positive Control should be added to the well at 25 m units/well. For instance, diluted positive control 1:20 with the enzyme dilution buffer, use 10 µL for 1 assay. Unused AKT1 Positive control should be stored at -70°C.

Product Size: Recombinant AKT1: 5 units/100 µL

Formulation: The AKT1 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.

Dilution: The AKT1 Positive control should be diluted with an enzyme dilution buffer to avoid inactivating the enzyme activity in low protein concentration condition. Enzyme dilution buffer: Mix 9-parts of Kinase buffer and 1-part of 10X BSA (100 µg/mL x 0.25 mL)

Source: Human AKT1 containing N-terminal GST-tag and C-terminal His-tag, expressed in sf9 cells.

Molecular Weight: AKT1 Positive Control demonstrates a double 92 kDa bands by SDS-PAGE analysis.

Purity: AKT1 Positive Control is greater than 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 (Whattman 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.

Related Products:
* AKT Assay/Inhibitor Screening Kit: Cat# CY-1168
* AKT2 Positive Control: Cat# CY-E1168-2

References:
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