EPHB2 (Human), Active

Recombinant protein expressed in Sf9 cells

Cat# CY-SPE22

Lot No. P177-2
5 µg 0.1 µg/µl

Background:

EPHB2 is a member of the Eph family of receptor tyrosine kinases that mediate neurodevelopmental processes such as boundary formation, axon guidance, vasculogenesis, and cell migration. EPHB2 has been shown to associate with the non-receptor tyrosine kinase Abl and activated EPHB2 causes tyrosine phosphorylation of Abl and regulates its ctivity (1). EPHB2 is overexressed in a number of tumors particularly glioblastoma and this increases glioma cell migration and invasion (2).

Product Description:

Recombinant human EPHB2 (570-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_004442.

Gene Aliases:

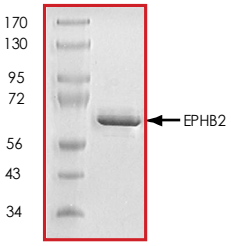
DRT, ERK, Hek5, EPHT3, Tyro5, CAPB, PCBC

Formulation:

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

Purity & Molecular Weight:

The purity was determined to be >90% by densitometry. Approx. MW 66kDa.



Storage:

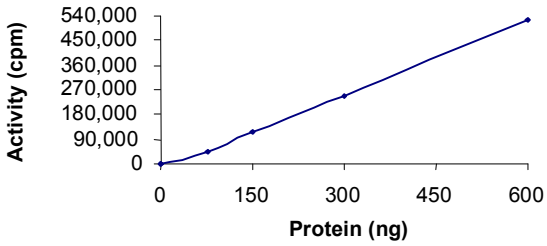
Store product at –70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

1 year at –70 °C from date of shipment.

Specific Activity:

The specific activity was determined to be 39 nmol /min/mg as per Activity Assay Protocol.



Activity Assay Protocol:

Assay activity of the kinase in a 25 µL reaction consisting of 5 µL of 5 X Kinase Assay Buffer, 10 µL of 1 mg/ml the Substrate Solution, 5 µL of diluted kinase and 5 µL of 250 µM ATP solution containing [gamma ^{32}P] ATP (0.167 µCi/µL). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20 µL of the reaction mixture onto phosphocellulose P81 paper. Air-dry the P81 paper and sequentially wash 4 times for approximately 10 minutes each in 1% phosphoric acid with constant gentle stirring. Count the P81 paper in a liquid scintillation counter.

Substrate Solution:

Poly (Glu:Tyr, 4:1) synthetic peptide substrate diluted in distilled H_2O to a final concentration of 1 mg/ml.

5 X Kinase Assay Buffer:

25mM MOPS, 12.5mM β-glycerol-phosphate, 20mM $MgCl_2$, 12.5mM $MnCl_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

References:

1.Yu, H.H. et al: Multiple signaling interactions of Abl and Arg kinases with the EphB2 receptor. Oncogene. 2001 Jul 5;20(30):3995-4006.

2.Nakada, M. et al: The phosphorylation of EphB2 receptor regulates migration and invasion of human glioma cells. Cancer Res. 2004 May 1;64(9):3179-85.

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