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EPHB1, Active

Recombinant protein expressed in Sf9 cells

Catalog # E21-11G

Lot # U268-1

Product Description

Recombinant mouse EPHB1 (591-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_173447.

Gene Aliases

Elk, Net, Cek6, Elkh, Hek6, EPHT2, AW488255, 9330129L11

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.

Storage and Stability

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.

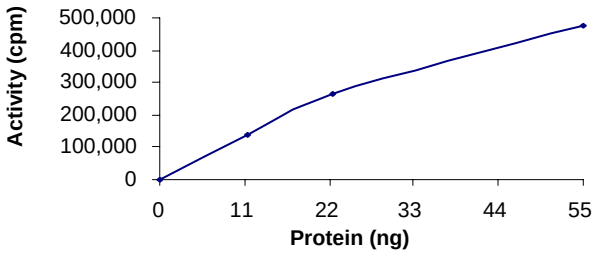
Scientific Background

EPHB1 is a member of the Eph family of receptor tyrosine kinases. Ligand-activated EPHB1 forms a signaling complex with Nck, paxillin, and focal adhesion kinase and induces tyrosine phosphorylation of paxillin in a c-Src-dependent manner to promote cell migration (1). In addition, activated EPHB1 recruits the adaptor proteins Grb2 and p52Shc and promotes p52Shc and c-Src tyrosine phosphorylation as well as MAPK/extracellular signal-regulated kinase (ERK) activation. Expression of dominant-negative c-Src significantly reduced EPHB1-dependent ERK1/2 activation and chemotaxis (2).

References

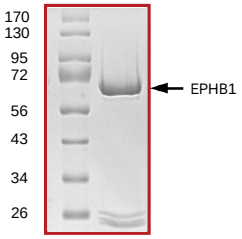
1. Vindis, C. et al: EphB1-mediated cell migration requires the phosphorylation of paxillin at Tyr-31/Tyr-118. J Biol Chem. 2004 Jul 2;279(27):27965-70.
2. Vindis, C. et al: EphB1 recruits c-Src and p52Shc to activate MAPK/ERK and promote chemotaxis. J Cell Biol. 2003 Aug 18;162(4):661-71.

Specific Activity



The specific activity of EPHB1 was determined to be **143 nmol/min/mg** as per activity assay protocol.

Purity



The purity of EPHB1 was determined to be **>90%** by densitometry, approx. MW **62kDa**.

EPHB1, Active

Recombinant protein expressed in Sf9 cells

Catalog Number	E21-11G
Specific Activity	143 nmol/min/mg
Specific Lot Number	U268-1
Purity	>90%
Concentration	0.1 µg/µl
Stability	1yr At –70°C from date of shipment
Storage & Shipping	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. Product shipped on dry ice.

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: E21-11G)

Active EPHB1 (0.1μg/μl) diluted with Kinase Dilution Buffer VII (Catalog #: K27-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active EPHB1 for optimal results).

Kinase Dilution Buffer VII (Catalog #: K27-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/μl BSA and 5% glycerol solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{32}P]-ATP Assay Cocktail

Prepare 250μM [^{32}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [^{32P}]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at –20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200μl aliquots at –20°C.

Substrate

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

Assay Protocol

Step 1. Thaw [^{32}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active EPHB1, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.

Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20μl:

- **Component 1.** 10μl of diluted Active EPHB1 (Catalog #E21-11G)
- **Component 2.** 5μl of 1mg/ml stock solution of substrate
- **Component 3.** 5μl of distilled H_2O

Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H_2O.

Step 5. Initiate the reaction by the addition of 5 μl [^{32}P]-ATP Assay Cocktail bringing the final volume up to 25μl and incubate the mixture in a water bath at 30°C for 15 minutes.

Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20 μl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H_2O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [P^{32}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μl [^{32}P]-ATP / pmoles of ATP (in 5 μl of a 250 μM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ^{32}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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