

Product Information

EPHB1, Active
mouse, recombinant
GST-tagged, expressed in Sf9 cells

Catalog Number **E7032**
Lot Number 019K0645
Storage Temperature $-70\text{ }^{\circ}\text{C}$

Synonyms: Elk; Net; Cek6; Elkh; Hek6; EPHT2;
AW488255; 9330129L11

Product Description

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

This recombinant product was expressed by baculovirus in Sf9 insect cells using an N-terminal GST-tag. The gene accession number is NM 173447. It is supplied in 50 mM Tris-HCl, pH 7.5, with 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~62 kDa

Purity: $\geq 70\%$ (SDS-PAGE, see Figure 1)

Specific Activity: 122–165 nmole/min/mg (see Figure 2)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product ships on dry ice and storage at $-70\text{ }^{\circ}\text{C}$ is recommended. After opening, aliquot into smaller quantities and store at $-70\text{ }^{\circ}\text{C}$. Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Lot Number 019K0645:
>90% (densitometry)

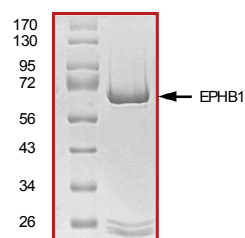
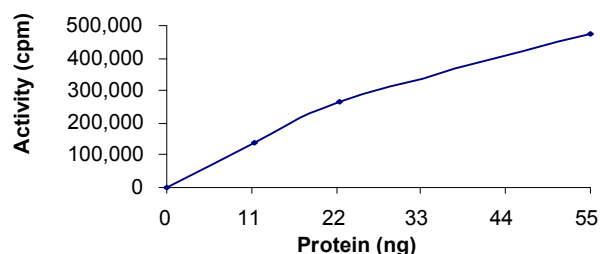


Figure 2.
Specific Activity of Lot Number 019K0645:
143 nmole/min/mg



Procedure

Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM MgCl_2 , 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with a 50 ng/ μl BSA and 5% glycerol solution.

Kinase Solution – Dilute the Active EPHB1 (0.1 µg/µl) with Kinase Dilution Buffer to the desired concentration. **Note:** The lot-specific specific activity plot may be used as a guideline (see Figure 2). It is recommended that the researcher perform a serial dilution of Active EPHB1 kinase for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200 µl aliquots at –20 °C.

γ-³²P-ATP Assay Cocktail (250 µM) – Combine 5.75 ml of Kinase Assay Buffer, 150 µl of 10 mM ATP Stock Solution, 100 µl of γ-³²P-ATP (1 mCi/100 µl). Store in 1 ml aliquots at –20 °C.

Substrate Solution – Dissolve the synthetic peptide substrate Poly(Glu:Tyr, 4:1) in water at a final concentration of 1 mg/ml.

1% phosphoric acid solution – Dilute 10 ml of concentrated phosphoric acid to a final volume of 1 L with water.

Kinase Assay

This assay involves the use of the ³²P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the Active EPHB1, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ-³²P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20 µl:
 - 10 µl of Kinase Solution
 - 5 µl of Substrate Solution
 - 5 µl of cold water (4 °C)
3. Set up a blank control as outlined in step 2, substituting 5 µl of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 µl of the γ-³²P-ATP Assay Cocktail, bringing the final reaction volume to 25 µl. Incubate the mixture in a water bath at 30 °C for 15 minutes.
5. After the 15 minute incubation, stop the reaction by spotting 20 µl of the reaction mixture onto an individually pre-cut strip of phosphocellulose P81 paper.

6. Air dry the pre-cut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of ~10 minutes each.
7. Set up a radioactive control to measure the total γ-³²P-ATP counts introduced into the reaction. Spot 5 µl of the γ-³²P-ATP Assay Cocktail on a pre-cut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity

Calculations:

1. Specific Radioactivity (SR) of ATP (cpm/nmole)

$$SR = \frac{\text{cpm of } 5 \mu\text{l of } \gamma\text{-}^{32}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

cpm – value from control (step 7)
nmole – 1.25 nmole (5 µl of 250 µM ATP Assay Cocktail)

2. Specific Kinase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta\text{cpm} \times (25/20)}{SR \times E \times T}$$

SR = specific radioactivity of the ATP (cpm/nmole ATP)
Δcpm = cpm of the sample – cpm of the blank (step 3)
25 = total reaction volume
20 = spot volume
T = reaction time (minutes)
E = amount of enzyme (mg)

References

1. Vindis, C. et al., EphB1-mediated cell migration requires the phosphorylation of paxillin at Tyr-31/Tyr-118. *J. Biol. Chem.*, **279**, 27965-27970 (2004).
2. Vindis, C. et al., EphB1 recruits c-Src and p52Shc to activate MAPK/ERK and promote chemotaxis. *J. Cell Biol.*, **162**, 661-671 (2003).

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