EPHB3 (585-end) Active human, recombinant
GST-tagged, expressed in Sf9 cells

Catalog Number E1158
Lot Number 019K1573
Storage Temperature –70 °C

Synonyms: ETK2; HEK2; Cek10; Mdk5; TYRO6

**Product Description**

EPHB3 is a member of the Ephrin receptor family and is expressed during embryonic development in multiple regions of the central nervous system. In the adult brain, EPHB3 is expressed in the cerebellum, raphe pallidus, hippocampus, entorhinal cortex, and both motor and sensory cortices.\(^1\) EPHB3 is involved in the maintenance of mature neuronal connections and/or rearrangement of synaptic connections during late stages of development. EPHB3 plays a role in the regulation of cell adhesion and migration, and the catalytic activity of EPHB3 is required for inhibition of integrin-mediated cell adhesion.\(^2\)

This recombinant product was expressed by baculovirus in Sf9 insect cells using an N-terminal GST-tag. The gene accession number is NM 004443. 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: \(\sim 68\) kDa

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

Specific Activity: 22–30 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 °C is recommended. After opening, aliquot into smaller quantities and store at –70 °C. Avoid repeated handling and multiple freeze/thaw cycles.

**Figure 1.**

SDS-PAGE Gel of Lot Number 019K1573:
\(\geq 90\%\) (densitometry)

**Figure 2.**

Specific Activity of Lot Number 019K1573:
26 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 EPHB3 (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 EPHB3 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.

$\gamma^{32}$P-ATP Assay Cocktail (250 µM) – Combine 5.75 ml of Kinase Assay Buffer, 150 µl of 10 mM ATP Stock Solution, 100 µl of $\gamma^{32}$P-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 $^{32}$P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the Active EPHB3, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The $\gamma^{32}$P-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 $\gamma^{32}$P-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 precut strip of phosphocellulose P81 paper.

6. Air dry the precut 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 $\gamma^{32}$P-ATP counts introduced into the reaction. Spot 5 µl of the $\gamma^{32}$P-ATP Assay Cocktail on a precut 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)
   \[
   SR = \frac{cpm \ of \ 5 \ µl \ of \ \gamma^{32}P-ATP \ Assay \ Cocktail}{nmole \ of \ ATP} \times \frac{nmole}{1.25 \ nmole} \times \frac{25}{20} \times \frac{E \times T}{SR} \times cpm \ – \ value \ from \ control \ (step \ 7)
   \]

2. Specific Kinase Activity (SA) (nmole/min/mg)
   \[
   nmole/min/mg = \frac{\Delta cpm \times (25/20)}{SR \times E \times T}
   \]

**References**


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