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Certificate of Analysis

EphB3, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-561, 14-561-K, 14-561M
Parent Lot # 33102U

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: *N*-terminal 6His-tagged recombinant human EphB3 residues 599–920, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose. Purity 93% by SDS-PAGE and Coomassie blue staining. MW = 40kDa.

Specific Activity (Parent lot# 33102U): 29U/mg, where one unit of EphB3, active activity is defined as 1nmol phosphate incorporated Into 0.1mg/ml poly(Glu, Tyr) (4:1) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 4.7mg/ml of enzyme in 50mM Tris/HCl pH7.5, 1mM EGTA, 150mM NaCl, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethnaol. Frozen solution.

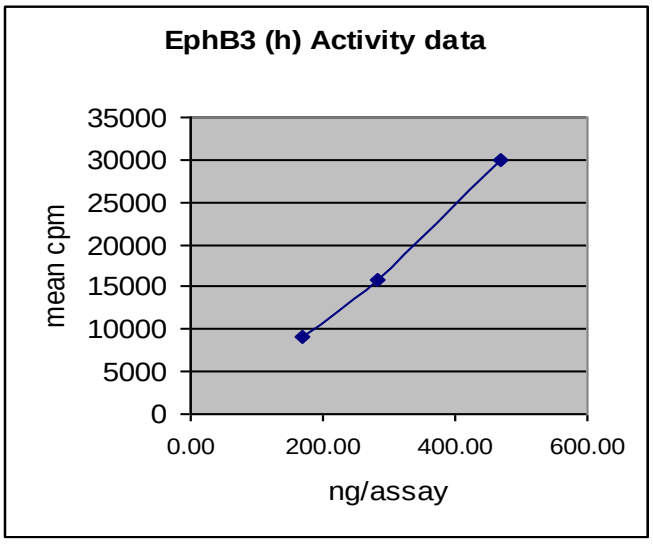
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

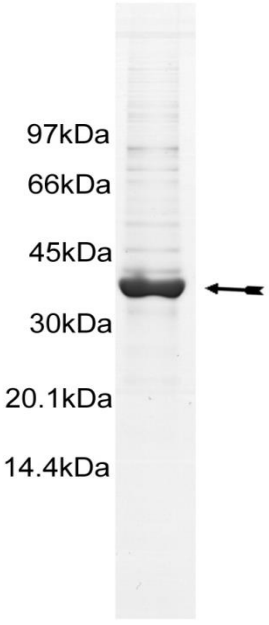
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 170–450ng of this lot of enzyme phosphorylated 0.1mg/ml poly(Glu, Tyr) (4:1) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as EphB3 with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of EphB3, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Manganese Chloride ($MnCl_2$):** Use at a final assay concentration of 10mM. Prepare a 200mM stock and add 1.25µl of stock per assay point.
3. **Poly(Glu, Tyr) (4:1) (PGT):** Use at a final assay concentration of 0.1mg/ml. Prepare a 1mg/ml stock and add 2.5µl of stock per assay point.
4. **EphB3, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 170–450ng per assay point.
5. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure

1. Add 5µl of 5 x reaction buffer to a microcentrifuge tube.
2. Add 2.5µl of **poly(Glu, Tyr) (4:1)**.
3. Add 1.25µl of $MnCl_2$.
4. Add 3.75µl of dH_2O.
5. Add **2.5µl (170–450ng) EphB3, active**.
6. Add 10µl of diluted [γ-^{33}P] ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
9. Transfer a 10µl aliquot onto the appropriate area of a **Filtermat A**.
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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EphB3 Sequence Information

Protein human EphB3

Tags *N*-terminal 6His

Native sequence Q29 of the recombinant protein is equivalent to Q599 of human EphB3

Accession number GenBank NM_004443

Recombinant EphB3 amino acid sequence:

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  1 MSYYHHHHHH DYDIPTTENL YFQGAMGSQY IAPGMKVYID PFTYEDPNEA VREFAKEIDV
 61 SCVKIEEVIG AGEFGEVCRG RLKQPGRREV FVAIKTLKVG YTERQRRDFL SEASIMGQFD
121 HPNIIRLEGV VTKSRPVMIL TEFMENCALD SFLRLNDGQF TVIQLVGMLR GIAAGMKYLS
181 EMNYVHRDLA ARNILVNSNL VCKVSDFGLS RFLEDDPSDP TYTSSLGGKI PIRWTAPEAI
241 AYRKFTSASD VWSYGIVMWE VMSYGERPYW DMSNQDVINA VEQDYRLPPP MDCPTALHQL
301 MLDCWVRDRN LRPKFSQIVN TLDKLIRNAA SLKVIASAQS GMSQPLLDRT
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Recombinant EphB3 nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatggg atcccagtac attgctcctg gaatgaaggt ttatattgac
 121 ccttttacct acgaggaccc taatgaggct gttcgggagt ttgccaagga gatcgacgtg
 181 tcctgcgtca agatcgagga ggtgatcgga gctggggaat ttggggaagt gtgccgtggt
 241 cgactgaaac agcctggccg ccgagaggtg tttgtggcca tcaagacgct gaaggtgggc
 301 tacaccgaga ggcagcggcg ggacttccta agcgaggcct ccatcatggg tcagtttgat
 361 caccccaata taatccggct cgagggcgtg gtcaccaaaa gtcggccagt tatgatcctc
 421 actgagttca tggaaaactg cgccctggac tccttcctcc ggctcaacga tgggcagttc
 481 acggtcatcc agctggtggg catgttgcgg ggcattgctg ccggcatgaa gtacctgtcc
 541 gagatgaact atgtgcaccg cgacctggct gctcgcaaca tccttgtcaa cagcaacctg
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 721 gcctatcgga agttcacttc tgctagtgat gtctggagct acggaattgt catgtgggag
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 841 gtggagcagg attaccggct gccaccaccc atggactgtc ccacagcact gcaccagctc
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 961 accctggaca agctcatccg caatgctgcc agcctcaagg tcattgccag cgctcagtct
1021 ggcatgtcac agcccctcct ggaccgcacg tag
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Reviewed and approved by site quality representative.