

Certificate of Analysis

EphB4, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-554, 14-554-K, 14-554M

Parent Lot # D7SN046U

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 EphB4, amino acids 561–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 96.8% by SDS-PAGE and Coomassie blue staining. MW = 51kDa.

Specific Activity (Parent lot# D7SN046U): 170U/mg, where one unit of EphB4, 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: 2.049mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

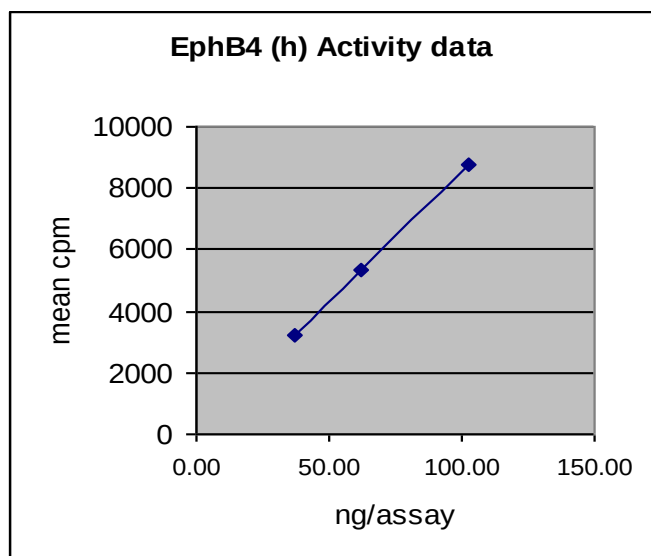
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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**

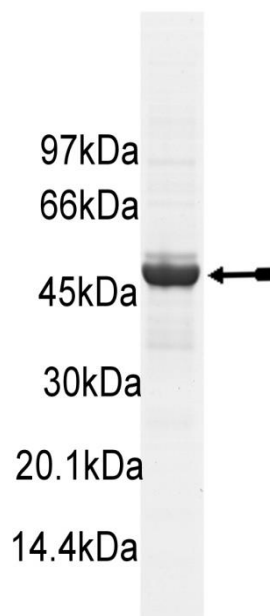
Quality Control Testing

Kinase Assay: 8–22ng 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 EphB4 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 EphB4, 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 (MnCl₂):** 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):** 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. **EphB4, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 8–22ng per assay point.
5. **[γ-³³P]ATP:** 2.5 x magnesium acetate/[γ-³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **poly(Glu, Tyr) (4:1)**.
3. Add 1.25µl of manganese chloride.
4. Add 3.75µl of dH₂O.
5. Add **2.5µl (8–22ng) EphB4, active**.
6. Add 10µl of diluted [γ-³³P] 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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EphB4 Sequence Information

<u>Protein</u>	Human EphB4
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	L31 of the recombinant protein is equivalent to L561 of human EphB4
<u>Accession number</u>	GenBank AY056047

Recombinant EphB4 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF LCLRKQSNR EAEYSDKHGQ YLIGHGTKVY
61 IDPFTYEDPN EAVREFAKEI DVSYVKIEEV IGAGEFGEVC RGRLKAPGKK ESCVAIKTLK
121 GGYTERQRRE FLSEASIMQ FEHPNIIRLE GVVTNMPPVM ILTEFMENGA LDSFLRLNDG
181 QFTVIQLVGM LRGIASGMRY LAEMSYVHRD LAARNILVNS NLVCKVSDFG LSRFLEENSS
241 DPTYTSSLGG KIPIRWTAPE AIAFRKFTSA SDAWSYGIVM WEVMSFGERP YWDSMNQDVI
301 NAIEQDYRLP PPPDCPTSLH QLMLDCWQKD RNARPRFPQV VSALDKMIRN PASLKIVARE
361 NGGASHPLLD QRQPHYSAFG SVGEWLRAIK MGRYEESFAA AGFGSFELVS QISAEDLLRI
421 GVTLAGHQKK ILASVQHMKs QAKPGTPGGT GGPAPQY

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Recombinant EphB4 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tatttttcagg gcgccatgga tccggaattc ctctgcctca ggaagcagag caatgggaga
121 gaagcagaat attcggacaa acacggacag tatctcatcg ggcatggtac taaggtctac
181 atcgaccctc tcacttatga agaccctaag gaggtgtgga gggaatttgc aaaagagatc
241 gatgtctcct acgtcaagat tgaagaggtg attggtgcag gtgagtttgg cgaggtgtgc
301 cggggggcggc tcaaggcccc agggagaaga gagagctgtg tggcaatcaa gacctgaag
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1201 gctggcttgg gctccttcga gctggtcagc cagatctctg ctgaggacct gctccgaatc
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1321 caggccaagc cgggaacccc ggtggtggca ggaggaccgg cccgcagta ctga

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