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Certificate of Analysis

EphB2, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-553, 14-553-K, 14-553M
Parent Lot # D8PN029U

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 EphB2 residues 560–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 79.3 % by SDS-PAGE and Coomassie blue staining. MW = 52kDa.

Specific Activity (Parent lot# D8PN029U): 153U/mg, where one unit of EphB2, 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: 2.56mg/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.

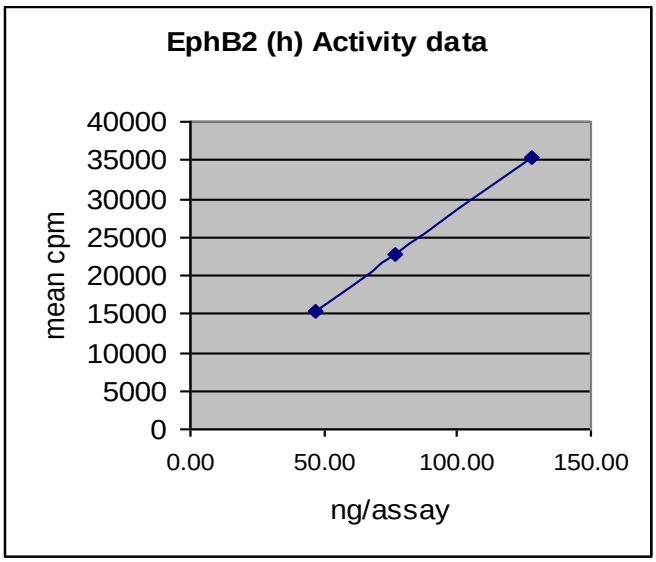
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

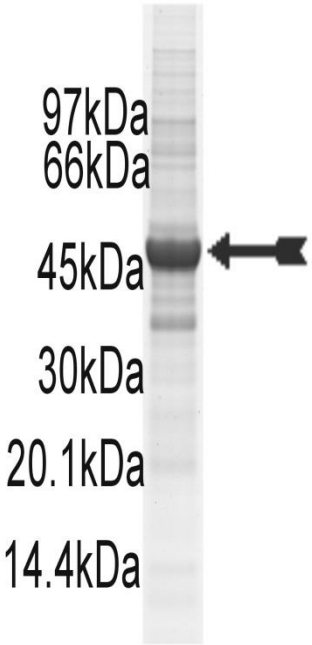
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 46–128ng of this lot of enzyme phosphorylated 0.1mg/ml poly(Glu, Tyr) 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 EphB2 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 EphB2, 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):** Use at a final assay concentration of 0.1 mg/ml. Prepare a 1mg/ml stock and add 2.5µl of stock per assay point.
4. **EphB2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 46–128ng 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 (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 dH_2O.
5. Add **2.5µl (46–128ng) EphB2, 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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EphB2 Sequence Information

Protein human EphB2

Tags *N*-terminal 6His

Native sequence C31 of the recombinant protein is equivalent to C560 of human EphB2

Accession number GenBank D31661. The recombinant protein is A848S and K918E with respect to this accession number. Both of these conflicts are reported in GenBank entries L36643, AF025304, NM_004442, NM_017449 and L41939.

Recombinant EphB2 amino acid sequence:

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  1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF CNRRGFERAD SEYTDKLQHY TSGHMTPGMK
 61 IYIDPFTYED PNEAVREFAK EIDISCVKIE QVIGAGEFGE VCSGHLKLPG KREIFVAIKT
121 LKSGYTEKQR RDFLSEASIM GQFDHPNVIH LEGVVTKSTP VMIITEFMEN GSLDSFLRQN
181 DGQFTVIQLV GMLRGIAAGM KYLADMNYVH RDLAARNILV NSNLVCKVSD FGLSRFLEDD
241 TSDPTYTSAL GGKIPIRWTA PEAIQYRKFT SASDVWSYGI VMWEVMSYGE RPYWDMTNQD
301 VINAIEQDYR LPPPMDCPSA LHQLMLDCWQ KDRNHRPKFG QIVNTLDKMI RNPNSLKAMA
361 PLSSGINLPL LDRTIPDYTS FNTVDEWLEA IKMGQYKESF ANAGFTSFDV VSQMMMEDIL
421 RVGVTLAGHQ KKILNSIQVM RAQMNQIQSV EV
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Recombinant EphB2 nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc tgtaacagac gggggtttga gcgtgctgac
 121 tcggagtaca cggacaagct gcaacactac accagtggcc acatgacccc aggcatgaag
 181 atctacatcg atcctttcac ctacgaggac cccaacgagg cagtgcggga gtttgccaag
 241 gaaattgaca tctcctgtgt caaaattgag caggtgatcg gagcagggga gtttggcgag
 301 gtctgcagtg gccacctgaa gctgccaggc aagagagaga tctttgtggc catcaagacg
 361 ctcaagtcgg gctacacgga gaagcagcgc cgggacttcc tgagcgaagc ctccatcatg
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 601 aagtacctgg cagacatgaa ctatgttcac cgtgacctgg ctgcccgcaa catcctcgtc
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 961 ctgcaccaac tcatgctgga ctgttggcag aaggaccgca accaccggcc caagttcggc
1021 caaattgtca acacgctaga caagatgatc cgcaatccca acagcctcaa agccatggcg
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1141 tttaacacgg tggacgagtg gctggaggcc atcaagatgg ggcagtacaa ggagagcttc
1201 gccaatgccg gcttcacctc ctttgacgtc gtgtctcaga tgatgatgga ggacattctc
1261 cgggttgggg tcactttggc tggccaccag aaaaaaatcc tgaacagtat ccaggtgatg
1321 cgggcgcaga tgaaccagat tcagtctgtg gaggtttga
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Reviewed and approved by site quality representative.