

Certificate of Analysis

EphB1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-674, 14-674-K, 14-674M

Parent Lot # 28383U

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

Product Description: N-terminal 6His-tagged, recombinant, human EphB1 amino acids 564–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 99% by SDS-PAGE and Coomassie blue staining. MW = 51.4kDa.

Specific Activity (Parent lot# 28383U): 206U/mg, where one unit of EphB1, active activity is defined as 1nmol phosphate incorporated into 250µM cdc2 peptide per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.069mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

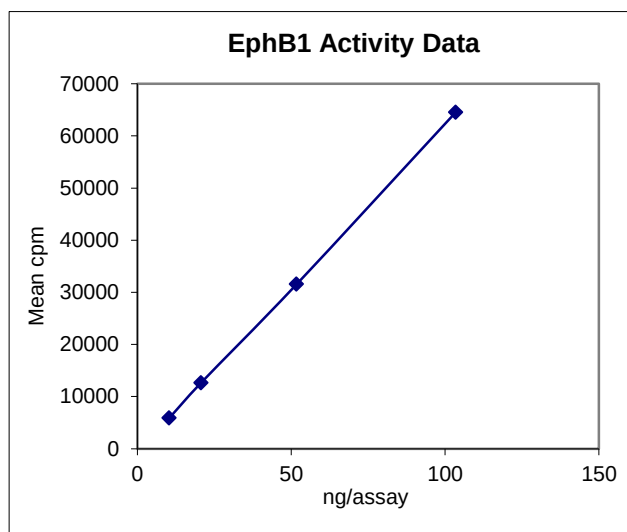
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

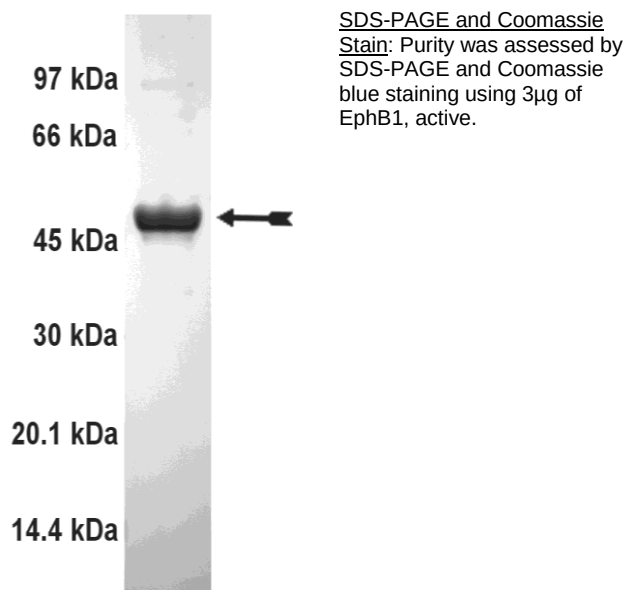
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 10–103ng of this lot of enzyme phosphorylated 250µM cdc2 peptide 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 EphB1 with the translated native sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **cdc2 peptide (KVEKIGEGTYGVVYK):** Use at a final assay concentration of 250µM. Prepare a 2.5mM stock and add 2.5µl of stock per assay point.
3. **EphB1, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 10–103ng per assay point.
4. **[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 **cdc2 peptide (KVEKIGEGTYGVVYK)**.
3. Add **2.5µl (10–103ng) EphB1, active**.
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ-³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. 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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EphB1 Sequence Information

Protein	human EphB1
Tags	N-terminal 6His
Native sequence	S31 of recombinant sequence is equivalent to S564 of native human EphB1
Accession number	GenBank NM_004441

Recombinant EphB1 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF SRKRAYSKEA VYSDKLQHYS TGRGSPGMKI
61 YIDPFTYEDP NEAVREFAKE IDVSFVKIEE VIGAGEFGEV YKGRCLKPGK REIYVAIKTL
121 KAGYSEKQRR DFLSEASIMG QFDHPNIIRL EGVVTKSRPV MIITEFMENG ALDSFLRQND
181 GQFTVIQLVG MLRGIAAGMK YLAEMNVVHR DLAARNILVN SNLVCKVSDF GLSRYLQDDT
241 SDPTYTSSLG GKIPVRWTAP EAIAYRKFTS ASDVWSYGIV MWEVMSFGER PYWDMNQDV
301 INAIEQDYRL PPPMDCPAAL HQLMLDCWQK DRNSRPRFAE IVNTLDKMIR NPASLKTIVAT
361 ITAVPSQPLL DRSIPDFTAF TTVDWLSAI KMQYRDSFL TAGFTSLQLV TQMTSEDLLR
421 IGITLAGHQK KILNSIHSR VQISQSPTAM A

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Recombinant EphB1 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc agcaggaaac gggcttatag caaagaggct
121 gtgtacagcg ataagctcca gcattacagc acaggccgag gctccccagg gatgaagatc
181 tacattgacc ctttacttta tgaggatccc aacgaagctg tccgggagtt tgccaaggag
241 attgatgtat cttttgtgaa aattgaagag gtcacgcggag caggggagtt tggagaagtg
301 tacaaggggc gtttgaact gccaggcaag agggaaatct acgtggccat caagaccctg
361 aaggcagggg actcggagaa gcagcgtcgg gactttctga gtgaggcgag catcatgggc
421 cagttcgacc atcctaaccat cattcgctg gaggggtgtg tcaccaagag tcggcctgtc
481 atgatcatca cagagttcat ggagaatggg gcattggatt ctttcctcag gcaaaatgac
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1201 actgctggct tcacctccct ccagctggct acccagatga catcagaaga cctcctgaga
1261 ataggcatca ccttggcagg ccatcagaag aagatcctga acagcattca ttctatgagg
1321 gtccagataa gtcagtcacc aacggcaatg gcatga

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