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Certificate of Analysis

EphA4, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-574, 14-574-K, 14-574M
Parent Lot # 28608U

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 EphA4 residues 60–892, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 97% by SDS-PAGE and Coomassie blue staining. MW = 37kDa.

Specific Activity (Parent lot# 28608U): 136U/mg, where one unit of EphA4 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: 1.939mg/ml of enzyme in 50mM Tris/HCl pH8.0, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

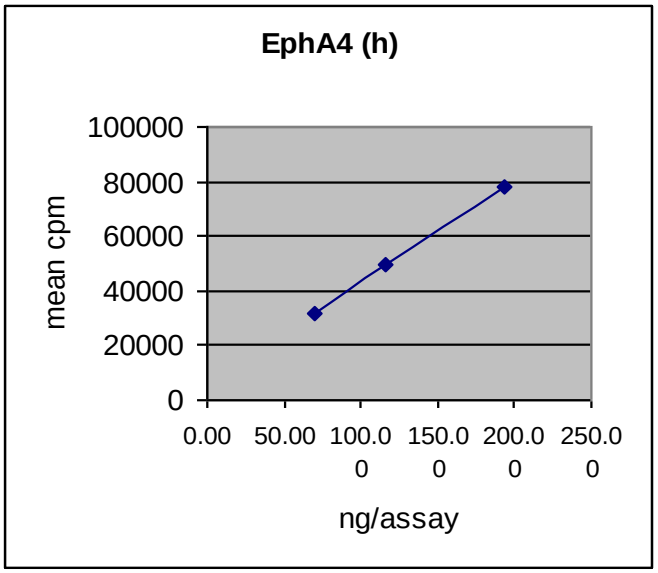
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

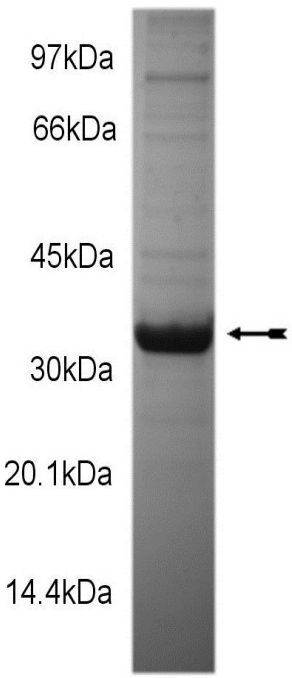
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 70–194ng 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 EphA4 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 EphA4, active.

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

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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 0.1mg/ml. Prepare a 1mg/ml stock and add 2µl of stock per assay point.
4. **EphA4, active:** Dilute in 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 70–194ng 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 (70–194ng) EphA4, 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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EphA4 Sequence Information

Protein human EphA4

Tags *N*-terminal 6His

Native sequence T31 of the fusion protein is equivalent to T601 of human EphA4

Accession number GenBank NM_004438

Recombinant EphA4 amino acid sequence:

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  1 MSYYHHHHHH DYDIPTTENL YFQGAMSLPR TYEDPNQAVR EFAKEIDASC IKIEKVIGVG
 61 EFGEVCSGRL KVPGKREICV AIKTLKAGYT DKQRRDFLSE ASIMGQFDHP NIIHLEGVVT
121 KCKPVMIITE YMENGSLDAF LRKNDGRFTV IQLVGMLRGI GSGMKYLSDM SYVHRDLAAR
181 NILVNSNLVC KVSDFGMSRV LEDDPEAAYT TRGGKIPIRW TAPEAIAYRK FTSASDVWSY
241 GIVMWEVMSY GERPYWDMSN QDVIKAIEEG YRLPPPMDCP IALHQLMLDC WQKERSDRPK
301 FGQIVNMLDK LIRNPNSLKR TG
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Recombinant EphA4 nucleotide sequence:

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  1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
 61 tattttcagg gcgccatgtc ccttccgcgg acgtacgaag atcccaacca agcagtgcga
121 gagtttgcca aagaaattga cgcatcctgc attaagattg aaaaagttat aggagttggt
181 gaatttggtg aggtatgcag tgggcgtctc aaagtgcctg gcaagagaga gatctgtgtg
241 gctatcaaga ctctgaaagc tggttataca gacaaacaga ggagagactt cctgagtgag
301 gccagcatca tgggacagtt tgaccatccg aacatcattc acttggaagg cgtggtcact
361 aaatgtaaac cagtaatgat cataacagag tacatggaga atggctcctt ggatgcattc
421 ctcaggaaaa atgatggcag atttacagtc attcagctgg tgggcatgct tcgtggcatt
481 gggtctggga tgaagtattt atctgatatg agctatgtgc atcgtgatct ggccgcacgg
541 aacatcctgg tgaacagcaa cttggtctgc aaagtgtctg attttggcat gtcccgagtg
601 cttgaggatg atccggaagc agcttacacc accaggggtg gcaagattcc tatccggtgg
661 actgcgccag aagcaattgc ctatcgtaaa ttcacatcag caagtgatgt atggagctat
721 ggaatcgtta tgtgggaagt gatgtcgtac ggggagaggc cctattggga tatgtccaat
781 caagatgtga ttaaagccat tgaggaaggc tatcggttac cccctccaat ggactgcccc
841 attgcgctcc accagctgat gctagactgc tggcagaagg agaggagcga caggcctaaa
901 tttgggcaga ttgtcaacat gttggacaaa ctcatccgca accccaacag cttgaagagg
961 acagggtag
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