eurofins | Discovery Services

Certificate of Analysis

EphA2, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-560, 14-560-K, 14-560M
Parent Lot # 28594U

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 EphA2 residues 596–900, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose. Purity 96% by SDS-PAGE and Coomassie blue staining. MW = 38kDa.

Specific Activity (Parent lot# 28594U): 268U/mg, where one unit of EphA2 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: 1.18mg/ml of enzyme in 50mM HEPES pH7.0, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

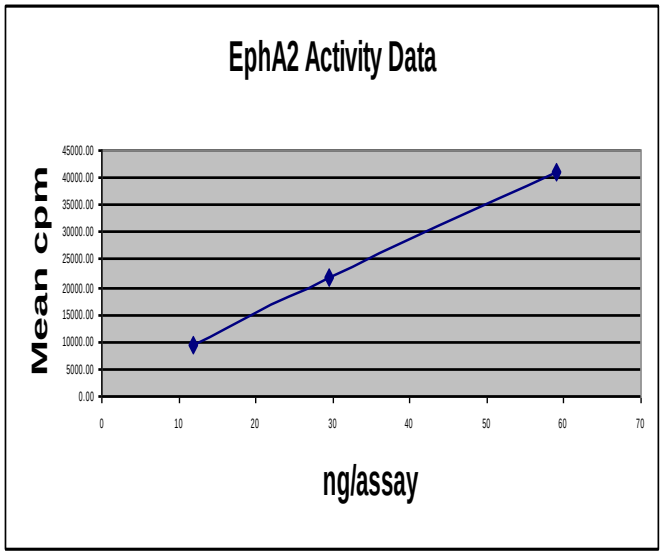
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

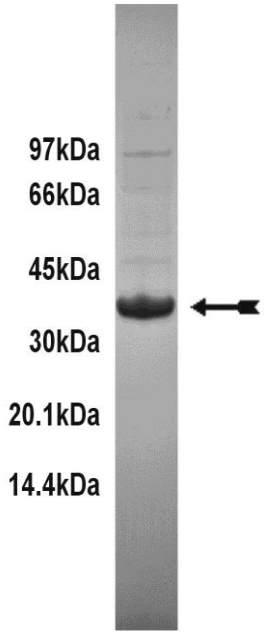
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 11.8–59ng 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 EphA2 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 active EphA2.

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

Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **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.
3. **EphA2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 11.8–59ng per assay point.
4. **[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 **2.5µl (11.8–59ng) EphA2, active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]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 **Filtermat A**.
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.

eurofins | Discovery Services

Certificate of Analysis

EphA2 Sequence Information

Protein human EphA2

Tags *N*-terminal 6His

Native sequence D29 of the recombinant protein is equivalent to D596 of human EphA2

Accession number GenBank NM_00431

Recombinant EphA2 amino acid sequence:

```
  1 MSYYHHHHHH DYDIPTTENL YFQGAMGSDP NQAVLKFTTE IHPSCVTRQK VIGAGEFGEV
 61 YKGMLKTSSG KKEVPVAIKT LKAGYTEKQR VDFLGEAGIM GQFSHHNIIR LEGVISKYKP
121 MMIITEYMEN GALDKFLREK DGEFSVLQLV GMLRGIAAGM KYLANMNYVH RDLAARNILV
181 NSNLVCKVSD FGLSRVLEDD PEATYTTSGG KIPIRWTAPE AISYRKFTSA SDVWSFGIVM
241 WEVMTYGERP YWELSNHEVM KAINDGFRLP TPMDCPSAIY QLMMQCWQQE RARRPKFADI
301 VSILDKLIRA PDSLKTLADF DPRVSIRLPS TSG
```

Recombinant EphA2 nucleotide sequence:

```
  1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
 61 tattttcagg gcgccatggg atctgacccc aaccaggctg tgttgaagtt cactaccgag
121 atccatccat cctgtgtcac tcggcagaag gtgatcggag caggagagtt tggggaggtg
181 tacaagggca tgctgaagac atcctcgggg aagaaggagg tgccggtggc catcaagacg
241 ctgaaagccg gctacacaga gaagcagcga gtggacttcc tcggcgaggc cggcatcatg
301 ggccagttca gccaccacaa catcatccgc ctagagggcg tcatctccaa atacaagccc
361 atgatgatca tcactgagta catggagaat ggggccctgg acaagttcct tcgggagaag
421 gatggcgagt tcagcgtgct gcagctggtg ggcatgctgc ggggcatcgc agctggcatg
481 aagtacctgg ccaacatgaa ctatgtgcac cgtgacctgg ctgcccgcaa catcctcgtc
541 aacagcaacc tggtctgcaa ggtgtctgac tttggcctgt cccgcgtgct ggaggacgac
601 cccgaggcca cctacaccac cagtggcggc aagatcccca tccgctggac cgccccggag
661 gccatttcct accggaagtt cacctctgcc agcgacgtgt ggagctttgg cattgtcatg
721 tgggaggtga tgacctatgg cgagcggccc tactgggagt tgtccaacca cgaggtgatg
781 aaagccatca atgatggctt ccggctcccc acacccatgg actgcccctc cgccatctac
841 cagctcatga tgcagtgctg gcagcaggag cgtgcccgcc gccccaagtt cgctgacatc
901 gtcagcatcc tggacaagct cattcgtgcc cctgactccc tcaagaccct ggctgacttt
961 gacccccgcg tgtctatccg gctccccagc acgagcggct ag
```

Reviewed and approved by site quality representative.