

Certificate of Analysis

EphA1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-653, 14-653-K, 14-653M

Parent Lot # WAD0136

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 EphA1, amino acids 568–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 87% by SDS-PAGE and Coomassie blue staining. MW = 50.6kDa.

Specific Activity (Parent lot# WAD0136): 48U/mg, where one unit of EphA1, 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: 1.39mg/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.

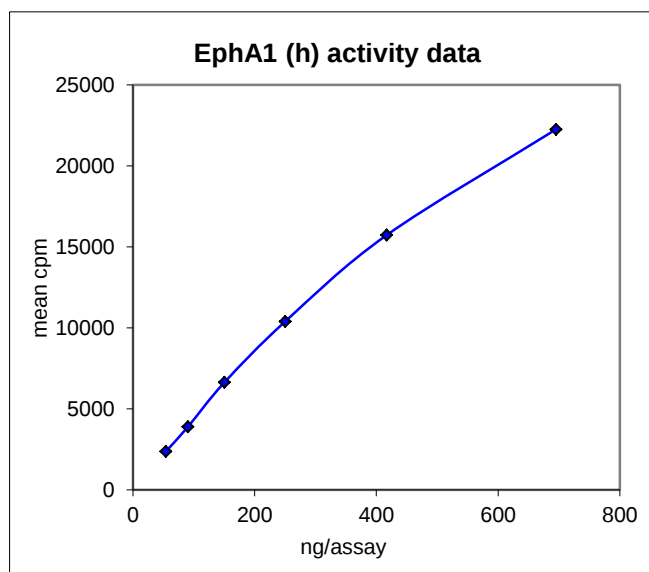
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

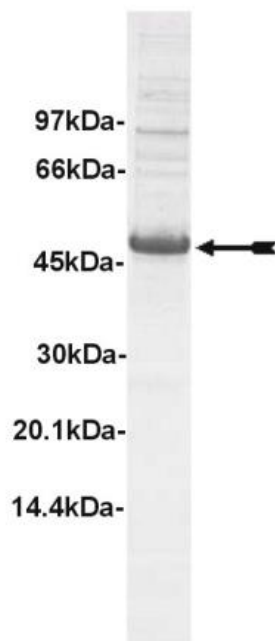
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 54–695ng 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 EphA1 with the translated native sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of EphA1, active.

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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 concentration of 0.1mg/ml. Make a 1mg/ml stock. Add 2.5µl of stock per assay point.
3. **EphA1, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 54–695ng 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 **poly(Glu, Tyr) (4:1)**.
3. Add **2.5µl (54–695ng) EphA1, 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 **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.

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EphA1 Sequence Information

Protein	Human EphA1
Tags	N-terminal 6His
Native sequence	F30 of the recombinant protein is equivalent to F568 of human EphA1
Accession number	Genbank AH007960

Recombinant EphA1 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF RSRRAQRQRQ QRQRDRATDV DREDKLWLKP
61 YVDLQAYEDP AQGALDFTRE LDPAWLMVDT VIGEGEFGEV YRGTLRRLPSQ DCKTVAIKTL
121 KDTSPGGQWW NFLREATIMG QFSHPHILHL EGVVTKRKPI MIITEFMENG ALDAFLRERE
181 DQLVPGQLVA MLQGIASGMN YLSNHNHYVHR DLAARNILVN QNLCKKVSDF GLTRLLDDFD
241 GTYETQGSKI PIRWTAPEAI AHRIFTTASD VWSFGIVMWE VLSFGDKPYG EMSNQEVMSK
301 IEDGYRLPPP VDCPAPLYEL MKNCWAYDRA RRPHFQKLQA HLEQLLANPH SLRTIANFDP
361 RVTLRRLPSLS GSDGIPYRTV SEWLESIRMK RYILHFHSAG LDTMECVLEL TAEDLTQMG
421 TLPGHQKRIL CSIQGFKD

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Recombinant EphA1 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgcatgga tccggaattc cggctcagga gagcccagcg gcagaggcag
121 cagaggcagc gtgaccgcgc caccgatgtg gatcgagagg acaagctgtg gctgaagcct
181 tatgtggacc tccaggcata cgaggaccct gcacaggagg ccttggactt taccggggag
241 cttgatccag cgtggctgat ggtggacact gtcataggag aaggagagtt tggggaagtg
301 tatcagggga ccctgaggct cccagccag gactgcaaga ctgtggccat taagacctta
361 aaagacacat cccagggtgg ccagtgggtg aacttccttc gagaggcaac tatcatgggc
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1261 aactgccccg ggcaccagaa gcgcattctt tgcagtattc agggattcaa ggactga

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Reviewed and approved by site quality representative.

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