

Certificate of Analysis

EphA3, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-644, 14-644-K, 14-644M

Parent Lot # WAE0367

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 EphA3 amino acids 578–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺-NTA agarose. Purity 77% by SDS-PAGE and Coomassie blue staining. MW = 49.0kDa.

Specific Activity (Parent lot# WAE0367): 91U/mg, where one unit of EphA3, 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: 1.19mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 10mM benzamidine, 1mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

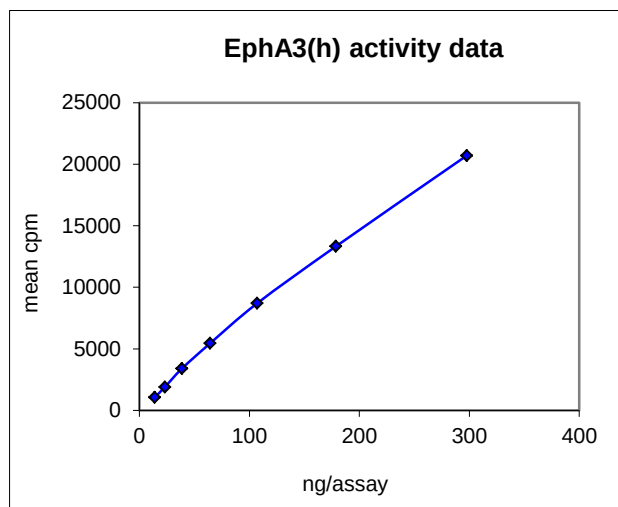
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

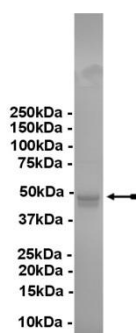
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 13.88–297.50ng 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 product identity as EphA3 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 EphA3, 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 assay concentration of 0.1mg/ml. Prepare a 1mg/ml stock. Add 2.5µl of stock per assay point.
3. **EphA3, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1M EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 13.88–297.50ng 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 (13.88–297.50ng) EphA3, 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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EphA3 Sequence Information

<u>Protein</u>	human EphA3
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	E31 of recombinant sequence is equivalent to E578 of native human EphA3
<u>Accession number</u>	GenBank NM_005233

Recombinant EphA3 amino acid sequence:

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1 MSYYHHHHH DYDIPTTENL YFQGAMSLGS EKRLHFGNGH LKLPGLRTYV DPHTYEDPTQ
61 AVHEFAKELD ATNISIDKVV GAGEFGEVCS GRLKLPSKKE ISVAIKTLKV GYTEKQRRDF
121 LGEASIMGQF DHPNIIRLEG VVTSKSPVMI VTEYMENGSL DSFLRKHDAQ FTVIQLVGML
181 RGIASGMKYL SDMGYVHRDL AARNILINSN LVCKVSDFGL SRVLEDDPEA AYTTTRGGKIP
241 IRWTSPEAIA YRKFTSASDV WSYGIVLWEV MSYGERPYWE MSNQDVIKAV DEGYRLPPPM
301 DCPAALYQLM LDCWQKDRNN RPKFEQIVSI LDKLIRNPGS LKIITSAAAR PSNLLLDQSN
361 VDITTFRTTG DWLNGVWTAH CKEIFTGVEY SSCDTIAKIS TDDMKKVGVT VVGPQKKIIS
421 SIKALETQSK NGPVPV

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Recombinant EphA3 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgtc ccttggatcc gaaaaaagac ttcatttttg caatgggcat
121 ttaaaacttc caggtctcag gacttatgtt gaccacata catatgaaga ccctacccaa
181 gctgttcatt agtttgcaa ggaattggat gccaccaaca tatccattga taaagttgtt
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301 atttcagtgg ccattaagac cctgaaagt ggctacacag aaaagcagag gagagacttc
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421 gttgttacca aaagtaagcc agttatgatt gtcacagaat acatggagaa tggttccttg
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1201 acagatgaca tgaaaaaggt tgggtgtcac gtggttgggc cacagaagaa gatcatcagt
1261 agcattaaag ctctagaaac gcaatcaaag aatggcccag ttcccgtgta g

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Reviewed and approved by site quality representative.

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