

Certificate of Analysis

EphA5, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-639, 14-639-K, 14-639M

Parent Lot # 1878307

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 EphA5, amino acids 655–956, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 89.3% by SDS-PAGE and Coomassie blue staining. MW = 37.9kDa.

Specific Activity (Parent lot# 1878307): 141U/mg, where one unit of EphA5, 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: 1.32mg/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.

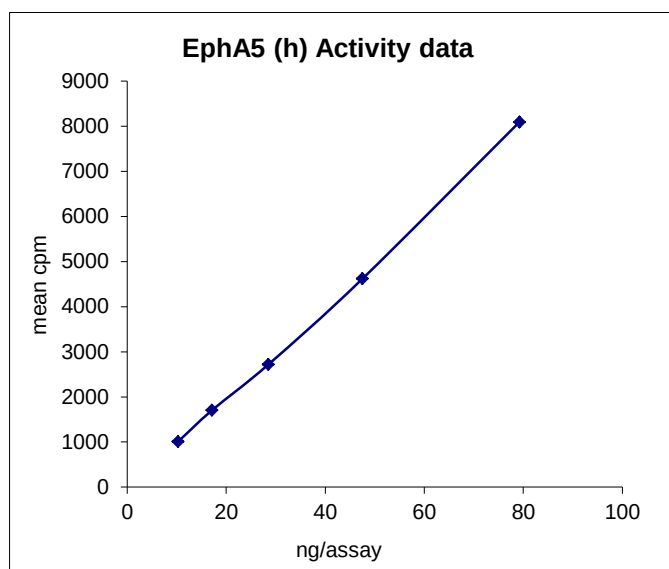
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

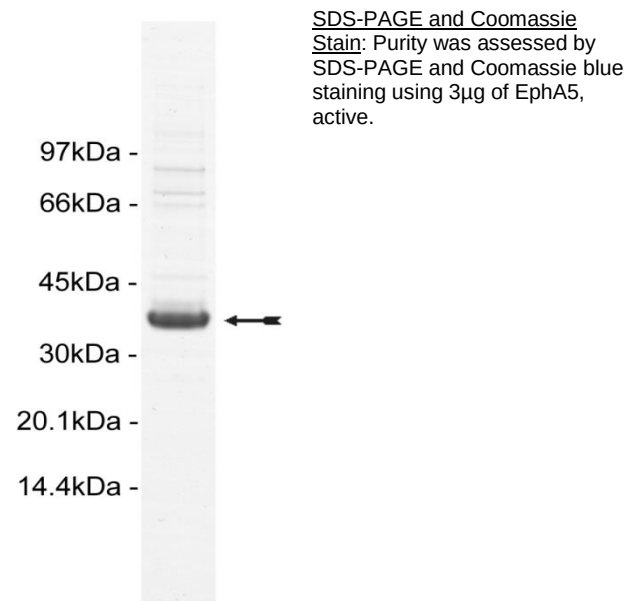
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 10–79ng 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 EphA5 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. **Manganese Chloride (MnCl₂):** 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 0.1 mg/ml. Prepare a 1mg/ml stock. Add 2.5 µl of stock per assay point.
4. **EphA5, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 10–79ng per assay point.
5. **[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 (10–79ng) EphA5, active**.
4. Add 2.5µl of 25mM MnCl₂.
5. Add 2.5µl of dH₂O.
6. Add 10µl of diluted [γ-³³P]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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EphA5 Sequence Information

<u>Protein</u>	Human EphA5
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	T31 of the recombinant protein is equivalent to T655 of human EphA5
<u>Accession number</u>	GenBank NM_004439

Recombinant EphA5 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF TYEDPNQAVH EFAKEIEASC ITIERVIGAG
61 EFGEVCSGRL KLP GKREL PV AIKTLKVG YT EKQRRDFLGE ASIMGQFDHP NIIHLEGVVT
121 KSKPVMIVTE YMENGSLDTF LKKNDGQFTV IQLVGMLRGI SAGMKYLSDM GYVHRDLAAR
181 NILINSNLVC KVSDFGLSRV LEDDPEAAYT TRGGKIPIRW TAPEAIAFRK FTSASDVWSY
241 GIVMWEVVS Y GERPYWEMTN QDVIKAVEEG YRLPSPMDCP AALYQLMLDC WQKERN SRPK
301 FDEIVNMLDK LIRNPSSLKT LVNASCRVSN LL

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Recombinant EphA5 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc acctatgagg atcccaatca agctgtccac
121 gaatttgcta aggagataga agcatcatgt atcaccattg agagagttat tggagcaggt
181 gaatttggtg aagtttgtag tggacgtttg aaactaccag gaaaaagaga attacctgtg
241 gctatcaaaa cccttaaaagt aggtataact gaaaagcaac gcagagattt cctaggtgaa
301 gcaagtatca tgggacagtt tgatcatcct aacatcatcc atttagaagg tgtggtgacc
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421 ttgaagaaaa acgatgggca gttcactgtg attcagcttg ttggcatgct gagaggatc
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901 tttgatgaaa tagtcaacat gttggacaag ctgatacgta acccaagtag tctgaagacg
961 ctggttaaat catcctgcag agtatctaatt ttattgtaa

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