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Certificate of Analysis

EphA8, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-673, 14-673-K, 14-673M
Parent Lot # 31069U

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 EphA8 residues 615–911, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}-NTA agarose. Purity 84% by SDS-PAGE and Coomassie blue staining. MW = 37.1kDa.

Specific Activity (Parent lot# 31069U): 152U/mg, where one unit of EphA8, active activity is defined as 1nmol phosphate incorporated into 100µM PDKtide per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.2mg/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.

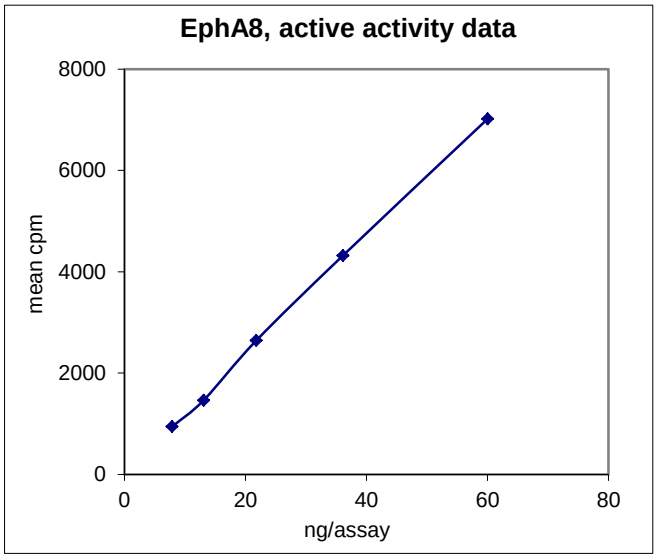
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

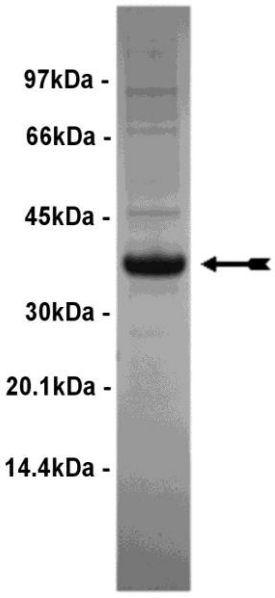
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 7.9–60ng of this lot of enzyme phosphorylated 100µM PDKtide 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 EphA8 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 EphA8, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **PDKtide (KTFCGTPEYLAPEVRREPRILSEEEQEMFRDFDYIADWC):** Use at a final assay concentration of 100µM. Prepare a 1mM stock. Add 2.5µl of stock per assay point.
3. **EphA8, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 7.9–60ng 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 **PDKtide (KTFCGTPEYLAPEVRREPRILSEEEQEMFRDFDYIADWC)**.
3. Add **2.5µl (7.9–60ng) EphA8, 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 **P30 Filtermat**.
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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EphA8 Sequence Information

Protein human EphA8

Tags *N*-terminal 6His

Native sequence T31 of recombinant sequence is equivalent to T615 of native human EphA8

Accession number GenBank NM_020526

Recombinant EphA8 amino acid sequence:

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  1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF TYEEPGRAGR SFTREIEASR IHIEKIIGSG
 61 DSGEVCYGRL RVPGQRDVPV AIKALKAGYT ERQRRDFLSE ASIMGQFDHP NIIRLEGVVT
121 RGRLAMIVTE YMENGSLDTF LRTHDGQFTI MQLVGMLRGV GAGMRYLSDL GYVHRDLAAR
181 NVLVDSNLVC KVSDFGLSRV LEDDPDAAYT TTGGKIPIRW TAPEAIAFRT FSSASDVWSF
241 GVVMWEVLAY GERPYWNMTN RDVISSVEEG YRLPAPMGCP HALHQLMLDC WHKDRAQRPR
301 FSQIVSVLDA LIRSPESLRA TATVSRC
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Recombinant EphA8 nucleotide sequence:

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  1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
 61 tattttcagg gcgccatgga tccggaattc acctacgagg agccaggccg ggcgggccgc
121 agtttcactc gggagatcga ggcctctagg atccacatcg agaaaatcat cggctctgga
181 gactccgggg aagtctgcta cgggaggctg cgggtgccag ggcagcggga tgtgcccgtg
241 gccatcaagg ccctcaaagc cggctacacg gagagacaga ggcgggactt cctgagcgag
301 gcgtccatca tggggcaatt cgaccatccc aacatcatcc gcctcgaggg tgtcgtcacc
361 cgtggccgcc tggcaatgat tgtgactgag tacatggaga acggctctct ggacaccttc
421 ctgaggaccc acgacgggca gttcaccatc atgcagctgg tgggcatgct gagaggagtg
481 ggtgccggca tgcgctacct ctcagacctg ggctatgtcc accgagacct ggccgcccgc
541 aacgtcctgg ttgacagcaa cctggtctgc aaggtgtctg acttcgggct ctcacgggtg
601 ctggaggacg acccggatgc tgcctacacc accacgggcg ggaagatccc catccgctgg
661 acggccccag aggccatcgc cttccgcacc ttctcctcgg ccagcgacgt gtggagcttc
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781 cgggatgtca tcagctctgt ggaggagggg taccgcctgc ccgcacccat gggctgcccc
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901 ttctcccaga ttgtcagtgt cctcgatgcg ctcatccgca gccctgagag tctcagggcc
961 accgccacag tcagcaggtg ctaa
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Reviewed and approved by site quality representative.