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Certificate of Analysis

EphA7, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-672
Lot # 1638877 **From bulk lot # D8KN008U**

Product Description: *N*-terminal 6His-tagged, recombinant human EphA7, amino acids 613–909, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 98% by SDS-PAGE and Coomassie blue staining. MW = 38kDa.

Specific Activity (lot# 1638877): 53U/mg, where one unit of EphA7, active activity is defined as 1nmol phosphate incorporated into 100µM PDKtide per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.774mg/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.

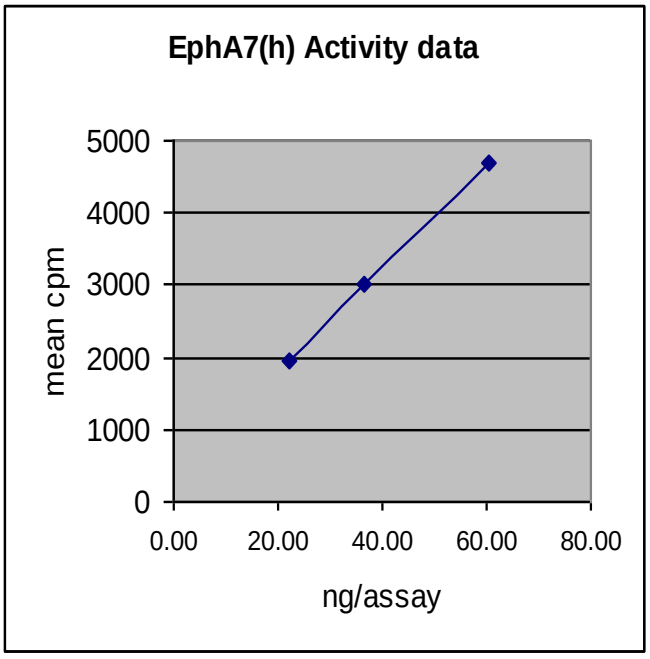
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

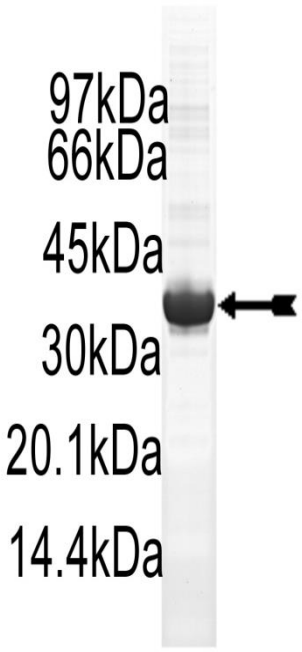
Quality Control Testing

Kinase Assay: 22.01–60.64ng 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 EphA7 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 EphA7, 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. **EphA7, active:** Dilute with 20mM MOPS-NaOH pH 7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 22.01–60.64ng 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 (22.01–60.64ng) EphA7, 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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EphA7 Sequence Information

Protein human EphA7

Tags *N*-Terminal 6His

Native sequence T37of the recombinant sequence is equivalent to T613 of native human EphA7

Accession number GenBank NM_004440

Recombinant EphA7 amino acid sequence:

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  1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQTYED PNRAVHQFAK ELDASCIKIE
 61 RVIGAGEFGE VCSGRLKLPG KRDVAVAIKT LKVGYTEKQR RDFLCEASIM GQFDHPNVVH
121 LEGVVTRGKP VMIVIEFMEN GALDAFLRKH DGQFTVIQLV GMLRGIAAGM RYLADMGYVH
181 RDLAARNILV NSNLVCKVSD FGLSRVIEDD PEAVYTTTGG KIPVRWTAPE AIQYRKFTSA
241 SDVWSYGIVM WEVMSYGERP YWDMSNQDVI KAIEEGYRLP APMDCPAGLH QLMLDCWQKE
301 RAERPKFEQI VGILDKMIRN PNSLKTPLGT CSR
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Recombinant EphA7 nucleotide sequence:

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  1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
 61 tattttcagg gcgccatgga tccggaattc aaaggcctac gtcgacagac ctatgaggac
121 ccaaatagag ctgtccatca attcgccaag gagctagatg cctcctgtat taaaattgag
181 cgtgtgattg gtgcaggaga attcggtgaa gtctgcagtg gccgtttgaa acttccaggg
241 aaaagagatg ttgcagtagc cataaaaacc ctgaaagttg gttacacaga aaaacaaagg
301 agagactttt tgtgtgaagc aagcatcatg gggcagtttg accacccaaa tgttgtccat
361 ttggaagggg ttgttacaag agggaaacca gtcatgatag taatagagtt catggaaaat
421 ggagccctag atgcatttct caggaaacat gatgggcaat ttacagtcat tcagttagta
481 ggaatgctga gaggaattgc tgctggaatg agatatttgg ctgatatggg atatgttcac
541 agggaccttg cagctcgcaa tattcttgtc aacagcaatc tcgtttgtaa agtgtcagat
601 tttggcctgt cccgagttat agaggatgat ccagaagctg tctatacaac tactggtgga
661 aaaattccag taaggtggac agcacccgaa gccatccagt accggaaatt cacatcagcc
721 agtgatgtat ggagctatgg aatagtcatg tgggaagtta tgtcttatgg agaaagacct
781 tattgggaca tgtcaaatca agatgttata aaagcaatag aagaaggtta tcgtttacca
841 gcacccatgg actgcccagc tggccttcac cagctaatgt tggattgttg gcaaaaggag
901 cgtgctgaaa ggccaaaatt tgaacagata gttggaattc tagacaaaat gattcgaaac
961 ccaaatagtc tgaaaactcc cctgggaact tgtagtaggt aa
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Reviewed and approved by site quality representative.