Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active EPH B1 [565 – 984]

Enzyme description:- EPH B1 [565 - 984]

Clone number:- DU 4455

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

Purification method:- Ni^{2+}-NTA agarose

Calculated molecular mass:-
Monoisotopic 51, 193.71 daltons
Average Mass 51, 226.50 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.33

Purity:- 80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2–mercaptoethanol, 10 mM MgAc

Substrate:-
Poly Glu Tyr (4:1) Final concentration: 0.1 mg/ml

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Clone Data Sheet

EPH B1 [565 - 984]

Protein	EPH B1 [565 - 984]
Clone number	DU 4455
Species	Human
Accession number	NM_004441
Tags	N-terminal His6
Bacterially expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMDPEF**RKRAYSKEAVYSDKLQHY STGRGSPGMKIYIDPFTYEDPNEAVREFAKEIDVSFVKIEEVIGAGEF GEVYKGRLKLPGKREIYVAIKTLKAGYSEKQRRDFLSEASIMGQFDHP NIIRLEGVVTKSRPVMIITEFMENGALDSFLRQNDGQFTVIQLVGMLR GIAAGMKYLAEMNYVHRDLAARNILVNSNLVCKVSDFGLSRYLQDDTS DPTYTSSLGGKIPVRWTAPEAIAYRKFTSASDVWSYGIVMWEVMSFGE RPYWDMSNQDVINAIEQDYRLPPPMDCPAALHQLMLDCWQKDRNSRPR FAEIVNTLDKMIRNPASLKTVATITAVPSQPLLDRSIPDFTAFTTVDD WLSAIKMVQYRDSFLTAGFTSLQLVTQMTSEDLLRIGITLAGHQKKIL NSIHSMRVQISQSPTAMA**
Native sequence	Amino acids R565 – A984 (end) of human EPH B1. Residue R31 of the fusion protein is equivalent to R565 of the native enzyme. The His(6) tag is located at residues 5 – 10.
Protease cleavage	rTEV (ENLYFQG) residues 18 - 24
Cloning sites	*Eco*R1 and *Not*1 sites of pFastBAC HTa

Nucleotide Sequence

ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAACG
ACCGAAAACCTGTATTTTCAGGGCGCCATGGATCCGGAATTC**AGGAAA**
CGGGCTTATAGCAAAGAGGCTGTGTACAGCGATAAGCTCCAGCATTAC
AGCACAGGCCGAGGCTCCCCAGGGATGAAGATCTACATTGACCCCTTC
ACTTACGAGGATCCCAACGAAGCTGTCCGGGAGTTTGCCAAGGAGATT
GATGTATCTTTTGTGAAAATTGAAGAGGTCATCGGAGCAGGGGAGTTT
GGAGAAGTGTACAAGGGGCGTTTGAAACTGCCAGGCAAGAGGGAAATC
TACGTGGCCATCAAGACCCTGAAGGCAGGGTACTCGGAGAAGCAGCGT
CGGGACTTTCTGAGTGAGGCGAGCATCATGGGCCAGTTCGACCATCCT
AACATCATTCGCCTGGAGGGTGTGGTCACCAAGAGTCGGCCTGTCATG
ATCATCACAGAGTTCATGGAGAATGGTGCATTGGATTCTTTCCTCAGG
CAAAATGACGGGCAGTTCACCGTGATCCAGCTTGTGGGTATGCTCAGG
GGCATCGCTGCTGGCATGAAGTACCTGGCTGAGATGAATTATGTGCAT
CGGGACCTGGCTGCTAGGAACATTCTGGTCAACAGTAACCTGGTGTGC
AAGGTGTCCGACTTTGGCCTCTCCCGCTACCTCCAGGATGACACCTCA
GATCCCACCTACACCAGCTCCTTGGGAGGGAAGATCCCTGTGAGATGG
ACAGCTCCAGAGGCCATCGCCTACCGCAAGTTCACTTCAGCCAGCGAC
GTTTGGAGCTATGGGATCGTCATGTGGGAAGTCATGTCATTTGGAGAG
AGACCCTATTGGGATATGTCCAACCAAGATGTCATCAATGCCATCGAG
CAGGACTACCGGCTGCCCCCACCCATGGACTGTCCAGCTGCTCTACAC
CAGCTCATGCTGGACTGTTGGCAGAAGGACCGGAACAGCCGGCCCCGG
TTTGCGGAGATTGTCAACACCCTAGATAAGATGATCCGGAACCCGGCA
AGTCTCAAGACTGTGGCAACCATCACCGCCGTGCCTTCCCAGCCCCTG
CTCGACCGCTCCATCCCAGACTTCACGGCCTTTACCACCGTGGATGAC
TGGCTCAGCGCCATCAAAATGGTCCAGTACAGGGACAGCTTCCTCACT
GCTGGCTTCACCTCCCTCCAGCTGGTCACCCAGATGACATCAGAAGAC
CTCCTGAGAATAGGCATCACCTTGGCAGGCCATCAGAAGAAGATCCTG
AACAGCATTCATTCTATGAGGGTCCAGATAAGTCAGTCACCAACGGCA
ATGGCAtgagcggccgc