Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active EPH B3 [561 – 998]

Enzyme description:- EPH B3 [561 - 998]

Clone number:- DU 4875

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal His(6)

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

Expression level:- 5 mg/L

Calculated molecular mass:-
Monoisotopic 52, 528.45 daltons
Average Mass 52, 562.31 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.03

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:- -80 °C

Assay:- Standard filter binding assay

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

Specific activity range:- To be determined

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Clone Data Sheet

EPH B3 [561 - 998]

Protein	EPH B3 [561 - 998]
Clone number	DU 4875
Species	Human
Accession number	NM_004443
Tags	N-terminal His6
Bacterially expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGS**VGSATAGLVFVVAVVVIAIV CLRKQRHGSDSEYTEKLQQYIAPGMKVYIDPFTYEDPNEAVREFAKEI DVSCVKIEEVIGAGEFGEVCRGRLKQPGRREVFVAIKTLKVGYTERQR RDFLSEASIMGQFDHPNIIRLEGVVTKSRPVMILTEFMENCALDSFLR LNDGQFTVIQLVGMLRGIAAGMKYLSEMNYVHRDLAARNILVNSNLVC KVSDFGLSRFLEDDPSDPTYTSSLGGKIPIRWTAPEAIAYRKFTSASD VWSYGIVMWEVMSYGERPYWDMSNQDVINAVEQDYRLPPPMDCPTALH QLMLDCWVRDRNLRPKFSQIVNTLDKLIRNAASLKVIASAQSGMSQPL LDRTVPDYTTFTTVGDWLDAIKMGRYKESFVSAGFASFDLVAQMTAED LLRIGVTLAGHQKKILSSIQDMRLQMNQTLPVQV**
Native sequence	Amino acids V561 – V998 (end) of human EPH B3. Residue V29 of the fusion protein is equivalent to V561 of the native enzyme. The His(6) tag is located at residues 5 – 10.
Protease cleavage	rTEV (ENLYFQG) residues 18 - 24
Cloning sites	*Bam*H1 and *Not*1 sites of pFastBAC HTb

Nucleotide Sequence

ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAACG
ACCGAAAACCTGTATTTTCAGGGCGCCATGGGATCC**GTGGGCTCCGCT**
ACAGCTGGGCTTGTCTTCGTGGTGGCTGTCGTGGTCATCGCTATCGTC
TGCCTCAGGAAGCAGCGACACGGCTCTGATTCGGAGTACACGGAGAAG
CTGCAGCAGTACATTGCTCCTGGAATGAAGGTTTATATTGACCCTTTT
ACCTACGAGGACCCTAATGAGGCTGTTCGGGAGTTTGCCAAGGAGATC
GACGTGTCCTGCGTCAAGATCGAGGAGGTGATCGGAGCTGGGGAATTT
GGGGAAGTGTGCCGTGGTCGACTGAAACAGCCTGGCCGCCGAGAGGTG
TTTGTGGCCATCAAGACGCTGAAGGTGGGCTACACCGAGAGGCAGCGG
CGGGACTTCCTAAGCGAGGCCTCCATCATGGGTCAGTTTGATCACCCC
AATATAATCCGGCTCGAGGGCGTGGTCACCAAAAGTCGGCCAGTTATG
ATCCTCACTGAGTTCATGGAAAACTGCGCCCTGGACTCCTTCCTCCGG
CTCAACGATGGGCAGTTCACGGTCATCCAGCTGGTGGGCATGTTGCGG
GGCATTGCTGCCGGCATGAAGTACCTGTCCGAGATGAACTATGTGCAC
CGCGACCTGGCTGCTCGCAACATCCTTGTCAACAGCAACCTGGTCTGC
AAAGTCTCAGACTTTGGCCTCTCCCGCTTCCTGGAGGATGACCCCTCC
GATCCTACCTACACCAGTTCCCTGGGCGGGAAGATCCCCATCCGCTGG
ACTGCCCCAGAGGCCATAGCCTATCGGAAGTTCACTTCTGCTAGTGAT
GTCTGGAGCTACGGAATTGTCATGTGGGAGGTCATGAGCTATGGAGAG
CGACCCTACTGGGACATGAGCAACCAGGATGTCATCAATGCCGTGGAG
CAGGATTACCGGCTGCCACCACCCATGGACTGTCCCACAGCACTGCAC
CAGCTCATGCTGGACTGCTGGGTGCGGGACCGGAACCTCAGGCCCAAA
TTCTCCCAGATTGTCAATACCCTGGACAAGCTCATCCGCAATGCTGCC
AGCCTCAAGGTCATTGCCAGCGCTCAGTCTGGCATGTCACAGCCCCTC
CTGGACCGCACGGTCCCAGATTACACAACCTTCACGACAGTTGGTGAT
TGGCTGGATGCCATCAAGATGGGGCGGTACAAGGAGAGCTTCGTCAGT
GCGGGGTTTGCATCTTTTGACCTGGTGGCCCAGATGACGGCAGAAGAC
CTGCTCCGTATTGGGGTCACCCTGGCCGGCCACCAGAAGAAGATCCTG
AGCAGTATCCAGGACATGCGGCTGCAGATGAACCAGACGCTGCCTGTG
CAGGTCtgagcggccgc