

Standard Operating Procedure

<u>Enzyme description:-</u>	EPH B2 [560 - 987]
<u>Clone number:-</u>	DU 4874
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6)
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose

Monoisotopic	51,900.62 daltons
Average Mass	51,934.27 daltons
[cysteines reduced, methionines have not been oxidised]	

Purity:- 80 %

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

50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc

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

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Clone Data Sheet

EPH B2 [560 - 987]

<u>Protein</u>	EPH B2 [560 - 987]
<u>Clone number</u>	DU 4874
<u>Species</u>	Human
<u>Accession number</u>	NM_004442
<u>Tags</u>	N-terminal His6
<u>Bacterially expressed protein</u>	MSYYHHHHHDYDIPTTENLYFQGAMGSVIAIVCNRRRGFERADSEYT DKLQHYTSGHMTPGMKIYIDPFTYEDPNEAVREFAKEIDISCVKIEQV IGAGEFGEVCSGHLKLPKREIFVAIKTLKSGYTEKQRRDFLSEASIM GQFDHPNVIHLEGVVTKSTPVMITEFMENGSLDSFLRQNDGQFTVIQ LVGMLRGIAAGMKYLADMNYVHRDLAARNILVNSNLVCKVSDFGLSRF LEDDTSDPTYTSALGGKIPIRWTAPEAIQYRKFTSASDVWSYGIVMWE VMSYGERPYWDMTNQDVINAIEQDYRLPPMDCPSALHQLMLDCWQKD RNHRPKFGQIVNTLDKMIRNPNSLKAMAPLSSGINLPLDRTIPDYTS FNTVDEWLEAIKMGQYKESFANAGFTSFDVVSQMMMEDILRVGVTLAG HQKKILNSIQVMRAQMNQIQSVEV
<u>Native sequence</u>	Amino acids V560 – V987 (end) of human EPH B2. Residue V29 of the fusion protein is equivalent to V560 of the native enzyme. The His(6) tag is located at residues 5 – 10.
<u>Protease cleavage</u>	rTEV (<u>ENLYFQG</u>) residues 18 - 24
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I sites of pFastBAC HTb

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Nucleotide Sequence

ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAACG
ACCGAAACCTGTATTTTCAGGGCGCCATGGGATCCGTCATCGCCATC
GTGTGTAACAGAAGACGGGGGTTTGAGCGTGCTGACTCGGAGTACACG
GACAAGCTGCAACACTACACCAGTGGCCACATGACCCCAGGCATGAAG
ATCTACATCGATCCTTTACCTACGAGGACCCCAACGAGGCAGTGCGG
GAGTTTGCCAAGGAAATTGACATCTCCTGTGTCAAAATTGAGCAGGTG
ATCGGAGCAGGGGAGTTTGGCGAGGTCTGCAGTGGCCACCTGAAGCTG
CCAGGCAAGAGAGAGATCTTTGTGGCCATCAAGACGCTCAAGTCGGGC
TACACGGAGAAGCAGCGCCGGGACTTCCTGAGCGAAGCCTCCATCATG
GGCCAGTTCGACCATCCCAACGTCATCCACCTGGAGGGTGTCGTGACC
AAGAGCACACCTGTGATGATCATCACCGAGTTCATGGAGAATGGCTCC
CTGGACTCCTTTCTCCGGCAAAACGATGGGCAGTTCACAGTCATCCAG
CTGGTGGGCATGCTTCGGGGCATCGCAGCTGGCATGAAGTACCTGGCA
GACATGAACTATGTTACCGTGACCTGGCTGCCCCGAACATCCTCGTC
AACAGCAACCTGGTCTGCAAGGTGTCGGACTTTGGGCTCTCACGCTTT
CTAGAGGACGATACCTCAGACCCACCTACACCAGTGCCCTGGGCGGA
AAGATCCCCATCCGCTGGACAGCCCCGGAAGCCATCCAGTACCGGAAG
TTCACCTCGGCCAGTGATGTGTGGAGCTACGGCATTGTCATGTGGGAG
GTGATGTCCTATGGGGAGCGGCCCTACTGGGACATGACCAACCAGGAT
GTAATCAATGCCATTGAGCAGGACTATCGGCTGCCACCGCCCATGGAC
TGCCCGAGCGCCCTGCACCAACTCATGCTGGACTGTTGGCAGAAGGAC
CGCAACCACCGGCCCAAGTTCGGCCAAATTGTCAACACGCTAGACAAG
ATGATCCGCAATCCCAACAGCCTCAAAGCCATGGCGCCCTCTCCTCT
GGCATCAACCTGCCGCTGCTGGACCGCACGATCCCCGACTACACCAGC
TTTAACACGGTGGACGAGTGGCTGGAGGCCATCAAGATGGGGCAGTAC
AAGGAGAGCTTCGCCAATGCCGGCTTCACCTCCTTTGACGTGCTGTCT
CAGATGATGATGGAGGACATCCTCCGGGTGGGGTCACTTTGGCTGGC
CACCAGAAAAAATCCTGAACAGTATCCAGGTGATGCGGGCGCAGATG
AACCAGATTCAGTCTGTGGAGGTTtgagcggccgc