

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active EPH B2 [560 – 987]

<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
<u>Expression level:-</u>	2 mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	51, 900.62 daltons
Average Mass	51, 934.27 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	5.77
<u>Purity:-</u>	80 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	
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	
<u>Storage temperature:-</u>	-70 °C
<u>Assay buffer:-</u>	
50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc	
<u>Substrate:-</u>	
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>	<p>MSYYHHHHHDYDIPTTENLYFQGGAMGSVIAIVCNRRRGFERADSEYTDKLGHYTSGHMTPGMKIYIDPFTYEDPNEAVREFAKEIDISCVKIEQVIGAGEFGEVCSGHLKLPKREIFVAIKTLKSGYTEKQRRDFLSEASIMGQFDHPNVIHLEGVVTKSTPVMII TEFMENGSLDSFLRQNDGQFTVIQLVGMLRGIAAGMKYLADMNYVHRDLAARNILVNSNLVCKVSDFGLSRFLLEDDTSDPTYTSALGGKIPIRWTAPEAIQYRKFTSASDVWSYGI VMWEVMSYGERPYWDMTNQDVINAIEQDYRLPPMDCPSALHQLMLDCWQKDRNHRPKFGQIVNTLDKMI RNPNSLKAMAPLSSGINLPLLDRTIPDYTSFNTVDEWLEAIKMGQYKESFANAGFTSFDVVSQMMMEDILRVGVTLAGHQKKILNSIQVMRAQMNQIQSVEV</p>
<u>Native sequence</u>	<p>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.</p>
<u>Protease cleavage</u>	rTEV (<u>ENLYFQG</u>) residues 18 - 24
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pFastBAC HTb

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Nucleotide
Sequence

ATGTCGTA CTACCATCACCATCACCATCACGATTACGATATCCCAACG
ACCGAAAACCTGTATTTTCAGGGCGCCATGGGATCCGTCATCGCCATC
GTGTGTAACAGAAGACGGGGGTTTGAGCGTGCTGACTCGGAGTACACG
GACAAGCTGCAACACTACACCAGTGGCCACATGACCCCAGGCATGAAG
ATCTACATCGATCCTTTACCTACGAGGACCCCAACGAGGCAGTGCGG
GAGTTTGCCAAGGAAATTGACATCTCCTGTGTCAA AATTGAGCAGGTG
ATCGGAGCAGGGGAGTTTGGCGAGGTCTGCAGTGGCCACCTGAAGCTG
CCAGGCAAGAGAGAGATCTTTGTGGCCATCAAGACGCTCAAGTCGGGC
TACACGGAGAAGCAGCGCCGGGACTTCCTGAGCGAAGCCTCCATCATG
GGCCAGTTCGACCATCCCAACGTCATCCACCTGGAGGGTGTCTGTGACC
AAGAGCACACCTGTGATGATCATCACCGAGTTCATGGAGAATGGCTCC
CTGGACTCCTTTCTCCGGCAAACGATGGGCAGTTCACAGTCATCCAG
CTGGTGGGCATGCTTCGGGGCATCGCAGCTGGCATGAAGTACCTGGCA
GACATGAACTATGTTACCGTGACCTGGCTGCCCGCAACATCCTCGTC
AACAGCAACCTGGTCTGCAAGGTGTCTGGACTTTGGGCTCTCACGCTTT
CTAGAGGACGATACCTCAGACCCACCTACACCAGTGCCCTGGGCGGA
AAGATCCCCATCCGCTGGACAGCCCCGGAAGCCATCCAGTACCGGAAG
TTCACCTCGGCCAGTGATGTGTGGAGCTACGGCATTGTCATGTGGGAG
GTGATGTCTATGGGGAGCGGCCCTACTGGGACATGACCAACCAGGAT
GTAATCAATGCCATTGAGCAGGACTATCGGCTGCCACCGCCCATGGAC
TGCCCGAGCGCCCTGCACCAACTCATGCTGGACTGTTGGCAGAAGGAC
CGCAACCACCGGCCAAGTTCGGCCAAATTGTCAACACGCTAGACAAG
ATGATCCGCAATCCCAACAGCCTCAAAGCCATGGCGCCCCTCTCCTCT
GGCATCAACCTGCCGCTGCTGGACCGCACGATCCCCGACTACACCAGC
TTTAACACGGTGGACGAGTGGCTGGAGGCCATCAAGATGGGGCAGTAC
AAGGAGAGCTTCGCCAATGCCGGCTTCACCTCCTTTGACGTCGTGTCT
CAGATGATGATGGAGGACATCCTCCGGGTGGGGTCACTTTGGCTGGC
CACCAGAAAAAATCCTGAACAGTATCCAGGTGATGCGGGCGCAGATG
AACCAGATTCAGTCTGTGGAGGTTtgagcggccgc