

Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active EPH B1 [565 – 984]

<u>Enzyme description:-</u>	EPH B1 [565 - 984]
<u>Clone number:-</u>	DU 4455
<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>Calculated molecular mass:-</u>	
Monoisotopic	51, 193.71 daltons
Average Mass	51, 226.50 daltons
[cysteines reduced, methionines have not been oxidised	
<u>Theoretical pI:-</u>	6.33
<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 B1 [565 - 984]

<u>Protein</u>	EPH B1 [565 - 984]
<u>Clone number</u>	DU 4455
<u>Species</u>	Human
<u>Accession number</u>	NM_004441
<u>Tags</u>	N-terminal His6
<u>Bacterially expressed protein</u>	MSYYHHHHHDYDIPPTENLYFQGAMDPEFRKRAYSKEAVYSDKLQHY STGRGSPGMKIYIDPFTYEDPNEAVREFAKEIDVSFVKIEEVIGAGEF GEVYKGRKLPGKREIYVAIKTLKAGYSEKQRRDFILSEASIMGQFDHP NIIRLEGVVTKSRPVMIITEFMENGALDSFLRQNDGQFTVIQLVGMLR GIAAGMKYLAEMNYVHRDLAARNILVNSNLVCKVSDFGLSRYLQDDTS DPTYTSSLGGKIPVRWTAPEAIAYRKFTSASDVWSYGVIVMWEVMSFGE R PYWDMSNQDVINAIEQDYRLPPPMDCPAALHQLMLDCWQKDRNSRPR FAEIVNTLDKMIRNPASLKTATITAVPSQPLLDRSIPDFTAFTVDD WLSAIKMVQYRDSFLTAGFTSLQLVTQMTSEDLLRIGITLAGHQKKIL NSIHSMRVQISQSPTAMA
<u>Native sequence</u>	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.
<u>Protease cleavage</u>	rTEV (<u>ENLYFQG</u>) residues 18 - 24
<u>Cloning sites</u>	<i>Eco</i> R1 and <i>Not</i> 1 sites of pFastBAC HTa

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Nucleotide
Sequence

ATGTCGTACTACCATTACCATCACCATCACGATTACGATATCCAACG
ACCGAAAACCTGTATTTCAGGGCGCCATGGATCCGAATT**CAGGAAA**
CGGGCTTATAGCAAAGAGGGCTGTGTACAGCGATAAGCTCCAGCATTAC
AGCACAGGCCGAGGCCTCCCCAGGGATGAAGATCTACATTGACCCCTTC
ACTTACGAGGATCCAACGAAGCTGTCCGGAGTTGCCAAGGGAGATT
GATGTATCTTGAAATTGAAGAGGTATCGGAGCAGGGAGTT
GGAGAAGTGTACAAGGGCGTTGAAACTGCCAGGCAAGAGGGAAATC
TACGTGCCATCAAGACCTGAAGGCAGGGTACTCGGAGAACGAGCGT
CGGGACCTTCTGAGTGAGGCAGCATCATGGCCAGTTGACCATCCT
AACATCATTGCTGGAGGGTGTGGTCACCAAGAGTCGGCTGTGAG
ATCATCACAGAGTTCATGGAGAATGGTGCATTGGATTCTTCCTCAGG
CAAAATGACGGGCAGTTCACCGTGTACCTGGCTGAGATGAATTATGTG
GGCATCGCTGCTGGCATGAAGTACCTGGCTGAGATGAATTATGTG
CGGGACCTGGCTGCTAGGAACATTCTGGTCAACAGTAACCTGGTGTGC
AAGGTGTCCGACTTGGCCTCTCCGCTACCTCCAGGATGACACCTCA
GATCCCACCTACACCAGCTCCTGGAGGGAAAGATCCCTGTGAGATGG
ACAGCTCCAGAGGCCATCGCCTACCGCAAGTTCACTTCAGCCAGCGAC
GTTTGGAGCTATGGATCGTCATGTGGAAAGTCATGTCATTGGAGAG
AGACCCATTGGATATGTCACCAAGATGTCAATGCCATCGAG
CAGGACTACCGGTGCCCAACCATGGACTGTCCAGCTGCTACAC
CAGCTCATGCTGGACTGTTGGCAGAAGGACCGGAACAGCCGGCCCCGG
TTTGCAGGAGATTGTCAACACCTAGATAAGATGATCCGAACCGGCA
AGTCTCAAGACTGTGGCAACCATACCGCCGTGCCTCCAGCCCCCTG
CTCGACCGCTCCATCCCAGACTTCACGGCCTTACCAACCGTGGATGAC
TGGCTAGGCCATCAAATGGTCCAGTACAGGGACAGCTCCTCCT
GCTGGCTTCACCTCCCTCAGCTGGTCACCCAGATGACATCAGAAGAC
CTCCTGAGAATAGGCATCACCTTGGCAGGCCATCAGAAGAAGATCCTG
AACAGCATTCAATTCTATGAGGGTCCAGATAAGTCAGTCACCAACGGCA
ATGGCAtgagcggccgc