

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active EPH A4 [570 – 986]

Enzyme description:- EPH A4 [570 - 986]

Clone number:- DU 4580

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 74, 567.72 daltons

Average Mass 74, 615.92 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.73

Purity:- 90 %

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

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Clone Data Sheet

EPH A4 [570 - 986]

<u>Protein</u>	EPH A4 [570 - 986]
<u>Clone number</u>	DU 4580
<u>Species</u>	Human
<u>Accession number</u>	NM_004438
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSPNSRVDSRRRSKYS KAKQEADEEKHLNQGVRTYVDPFTYEDPNQAVREFAKEIDASCIKIEKV IGVGEFGEVCSGRLKVP GKREICVAIKTLKAGYTDKQRRDFLSEASIMG QFDHPNIIHLEGVVTCKKPVMIITEYMENGSLDAFLRKNDGSFTVIQLV GMLRGIGSGMKYLS DMSYVHRDLAARNILVNSNLVCKVSDFGMSRVLED DPEAAYTTRGGKIPIRWTAPEAIA YRKFTSASDVWSYGI VMWEVMSYGE RPYWDMSNQDVIKAIIEGYRLPPPMDCPIALHQLMLDCWQKERSDRPKF GQIVNMLDKLIRNPNSLKR TGTSSRPNTALLDPSSPEFSAVVSVGDWL QAIKMDRYKDNFTAAGYTTLEAVVHVNQEDLARIGITAI THQNKILSSV QAMRTQMQQMHGR MVPV
<u>Native sequence</u>	Amino acids S570 – V986 (end) of human EPH A4. Residue S238 of the fusion protein is equivalent to S570 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 229
<u>Cloning sites</u>	<i>Sal</i> I and <i>Not</i> I site of pGEX 6P-3

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Nucleotide Sequence

ATGTCCCCTATACTAGGTTATTGGAAAAATTAAGGGCCTTGTGCAACCCA
CTCGACTTCTTTTGGAAATATCTTGAAGAAAAATATGAAGAGCATTTGTA
TGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGT
TTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAA
CACAGTCTATGGCCATCATACTGTTATATAGCTGACAAGCACAAACATGTT
GGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCG
GTTTTGGATATTAGATACGGTGTTCGAGAATTGCATATAGTAAAGACT
TTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAA
AATGTTTGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCAT
GTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTAT
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AAAACGTATTGAAGCTATCCACAAATTGATAAGTACTTGAAATCCAGC
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