Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active EPH B4 [561 – 987]

Enzyme description:- EPH B4 [561 - 987]

Clone number:- DU 4864

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal His(6) tag

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

Calculated molecular mass:-
Monoisotopic 50, 954.36 daltons
Average Mass 50, 986.91 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.44

Purity:- 85 %

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 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 B4 [561 - 987]

Protein	EPH B4 [561 - 987]
Clone number	DU 4864
Species	Human
Accession number	NP_004435.3
Tags	N-terminal His(6)
Bacterially expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGS**LCLRKQSNGREAEYSDKHGQY LIGHGTKVYIDPFTYEDPNEAVREFAKEIDVSYVKIEEVIGAGEFGEVC RGRLKAPGKKESCVAIKTLKGGYTERQRREFLSEASIMGQFEHPNIIRL EGVVTNSMPVMILTEFMENGALDSFLRLNDGQFTVIQLVGMLRGIASGM RYLAEMSYVHRDLAARNILVNSNLVCKVSDFGLSRFLEENSSDPTYTSS LGGKIPIRWTAPEAIAFRKFTSASDAWSYGIVMWEVMSFGERPYWDMSN QDVINAIEQDYRLPPPPDCPTSLHQLMLDCWQKDRNARPRFPQVVSALD KMIRNPASLKIVARENGGASHPLLDQRQPHYSAFGSVGEWLRAIKMGRY EESFAAAGFGSFELVSQISAEDLLRIGVTLAGHQKKILASVQHMKSQAK PGTPGGTGGPAPQY**
Native sequence	Amino acids L561 – Y987 (end) of human EPH B4. Residue L29 of fusion protein is equilivalent to L561 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*1site of pFBHTb

Nucleotide sequence of insert

ggatccCTCTGCCTCAGGAAGCAGAGCAATGGGAGAGAAGCAGAATATT
CGGACAAACACGGACAGTATCTCATCGGACATGGTACTAAGGTCTACAT
CGACCCCTTCACTTATGAAGACCCTAATGAGGCTGTGAGGGAATTTGCA
AAAGAGATCGATGTCTCCTACGTCAAGATTGAAGAGGTGATTGGTGCAG
GTGAGTTTGGCGAGGTGTGCCGGGGGCGGCTCAAGGCCCCAGGGAAGAA
GGAGAGCTGTGTGGCAATCAAGACCCTGAAGGGTGGCTACACGGAGCGG
CAGCGGCGTGAGTTTCTGAGCGAGGCCTCCATCATGGGCCAGTTCGAGC
ACCCCAATATCATCCGCCTGGAGGGCGTGGTCACCAACAGCATGCCCGT
CATGATTCTCACAGAGTTCATGGAGAACGGCGCCCTGGACTCCTTCCTG
CGGCTAAACGACGGACAGTTCACAGTCATCCAGCTCGTGGGCATGCTGC
GGGGCATCGCCTCGGGCATGCGGTACCTTGCCGAGATGAGCTACGTCCA
CCGAGACCTGGCTGCTCGCAACATCCTAGTCAACAGCAACCTCGTCTGC
AAAGTGTCTGACTTTGGCCTTTCCCGATTCCTGGAGGAGAACTCTTCCG
ATCCCACCTACACGAGCTCCCTGGGAGGAAAGATTCCCATCCGATGGAC
TGCCCCGGAGGCCATTGCCTTCCGGAAGTTCACTTCCGCCAGTGATGCC
TGGAGTTACGGGATTGTGATGTGGGAGGTGATGTCATTTGGGGAGAGGC
CGTACTGGGACATGAGCAATCAGGACGTGATCAATGCCATTGAACAGGA
CTACCGGCTGCCCCCGCCCCCAGACTGTCCCACCTCCCTCCACCAGCTC
ATGCTGGACTGTTGGCAGAAAGACCGGAATGCCCGGCCCCGCTTCCCCC
AGGTGGTCAGCGCCCTGGACAAGATGATCCGGAACCCCGCCAGCCTCAA
AATCGTGGCCCGGGAGAATGGCGGGGCCTCACACCCTCTCCTGGACCAG
CGGCAGCCTCACTACTCAGCTTTTGGCTCTGTGGGCGAGTGGCTTCGGG
CCATCAAAATGGGAAGATACGAAGAAAGTTTCGCAGCCGCTGGCTTTGG
CTCCTTCGAGCTGGTCAGCCAGATCTCTGCTGAGGACCTGCTCCGAATC
GGAGTCACTCTGGCGGGACACCAGAAGAAAATCTTGGCCAGTGTCCAGC
ACATGAAGTCCCAGGCCAAGCCGGGAACCCCGGGTGGGACAGGAGGACC
GGCCCCGCAGTACtgagcggccgc