Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active EPHA1 [568 – 976]

Enzyme description:- EPH A1 [568 - 976]

Clone number:- DU 63274

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 74, 059.48 daltons
Average Mass 74, 107.19 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.96

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:- 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:-
EFPIYDFLPAKKK Final concentration: 300 uM

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Clone Data Sheet

EPHA1 [568 - 976]

Protein	EPH A1 [568 - 976]
Clone number	DU 63274
Species	Human
Accession number	NM_005232.5
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEF**FRSRRAQRQRQ QRQRDRATDVDREDKLWLKPYVDLQAYEDPAQGALDFTRELDPAWLMVD TVIGEGEFGEVYRGTLRLPSQDCKTVAIKTLKDTSPGGQWWNFLREATI MGQFSHPHILHLEGVVTKRKPIMIITEFMENGALDAFLREREDQLVPGQ LVAMLQGIASGMNYLSNHNYVHRDLAARNILVNQNLCCKVSDFGLTRLL DDFDGTYETQGGKIPIRWTAPEAIAHRIFTTASDVWSFGIVMWEVLSFG DKPYGEMSNQEVMKSIEDGYRLPPPVDCPAPLYELMKNCWAYDRARRPH FQKLQAHLEQLLANPHSLRTIANFDPRVTLRLPSLSGSDGIPYRTVSEW LESIRMKRYILHFHSAGLDTMECVLELTAEDLTQMGITLPGHQKRILCS IQGFKD**

Native sequence	Amino acids F568 – D976 (end) of human EPH A1. Residue F235 of the fusion protein is equivalent to F568 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGPL) residues 221 - 229
Cloning sites	*Eco*R1 and *Not*1 site of pGEX 6P-1

Nucleotide Sequence Of Insert

gaattcTTCCGGTCCAGGAGAGCCCAGCGGCAGAGGCAGCAGAGGCAGC
GTGACCGCGCCACCGATGTGGATCGAGAGGACAAGCTGTGGCTGAAGCC
TTATGTGGACCTCCAGGCATACGAGGACCCTGCACAGGGAGCCCTGGAC
TTTACCCGGGAGCTTGATCCAGCGTGGCTGATGGTGGACACTGTCATAG
GAGAAGGAGAGTTTGGGGAAGTGTATCGAGGGACCCTGAGGCTCCCCAG
CCAGGACTGCAAGACTGTGGCCATTAAGACCTTAAAAGACACATCCCCA
GGTGGCCAGTGGTGGAACTTCCTTCGAGAGGCAACTATCATGGGCCAGT
TTAGCCACCCGCATATTCTGCATCTGGAAGGTGTCGTCACAAAGCGAAA
GCCGATCATGATCATCACAGAATTTATGGAGAATGGAGCCCTGGATGCC
TTCCTGAGGGAGCGGGAGGACCAGCTGGTCCCTGGGCAGCTAGTGGCCA
TGCTGCAGGGCATAGCATCTGGCATGAACTACCTCAGTAATCACAATTA
TGTCCACCGGGACCTGGCTGCCAGAAACATCTTGGTGAATCAAAACCTG
TGCTGCAAGGTGTCTGACTTTGGCCTGACTCGCCTCCTGGATGACTTTG
ATGGCACATACGAAACCCAGGGAGGAAAGATCCCTATCCGTTGGACAGC
CCCTGAAGCCATTGCCCATCGGATCTTCACCACAGCCAGCGATGTGTGG
AGCTTTGGGATTGTGATGTGGGAGGTGCTGAGCTTTGGGGACAAGCCTT
ATGGGGAGATGAGCAATCAGGAGGTTATGAAGAGCATTGAGGATGGGTA
CCGGTTGCCCCCTCCTGTGGACTGCCCTGCCCCTCTGTATGAGCTCATG
AAGAACTGCTGGGCATATGACCGTGCCCGCCGGCCACACTTCCAGAAGC
TTCAGGCACATCTGGAGCAACTGCTTGCCAACCCCCACTCCCTGCGGAC
CATTGCCAACTTTGACCCCAGGGTGACTCTTCGCCTGCCCAGCCTGAGT
GGCTCAGATGGGATCCCGTATCGAACCGTCTCTGAGTGGCTCGAGTCCA
TACGCATGAAACGCTACATCCTGCACTTCCACTCGGCTGGGCTGGACAC
CATGGAGTGTGTGCTGGAGCTGACCGCTGAGGACCTGACGCAGATGGGA
ATCACACTGCCCGGGCACCAGAAGCGCATTCTTTGCAGTATTCAGGGAT
TCAAGGACtgagcggccgc