Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active EPHA5 [595 – 1037]

Enzyme description:- EPH A5 [595 - 1037]

Clone number:- DU 63279

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 76, 764.69 daltons
Average Mass 76, 814.71 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.15

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

Division of Signal Tranduction Therapy

Clone Data Sheet

EPHA5 [595 - 1037]

Protein	EPH A5 [595 - 1037]
Clone number	DU 63279
Species	Human
Accession number	NM_004439.7
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEF**SGSCCECGCGR** **ASSLCAVAHPSLIWRCGYSKAKQDPEEEKMHFHNGHIKLPGVRTYIDPH** **TYEDPNQAVHEFAKEIEASCITIERVIGAGEFGEVCSGRLKLPGKRELP** **VAIKTLKVGYTEKQRRDFLGEASIMGQFDHPNIIHLEGVVTKSKPVMIV** **TEYMENGSLDTFLKKNDGQFTVIQLVGMLRGISAGMKYLSDMGYVHRDL** **AARNILINSNLVCKVSDFGLSRVLEDDPEAAYTTRGGKIPIRWTAPEAI** **AFRKFTSASDVWSYGIVMWEVVSYGERPYWEMTNQDVIKAVEEGYRLPS** **PMDCPAALYQLMLDCWQKERNSRPKFDEIVNMLDKLIRNPSSLKTLVNA** **SCRVSNLLAEHSPLGSGAYRSVGEWLEAIKMGRYTEIFMENGYSSMDAV** **AQVTLEDLRRLGVTLVGHQKKIMNSLQEMKVQLVNGMVPL**
Native sequence	Amino acids S595 – L1037 (end) of human EPH A5. Residue S235 of the fusion protein is equivalent to S595 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*1site of pGEX 6P-1

Nucleotide Sequence Of Insert

gaattcAGTGGAAGTTGCTGCGAATGTGGCTGTGGGAGGGCTTCTTCCC
TGTGCGCTGTTGCCCATCCAAGCCTAATATGGCGGTGTGGCTACAGCAA
AGCAAAACAAGATCCAGAAGAGGAAAAGATGCATTTTCATAATGGGCAC
ATTAAACTGCCAGGAGTAAGAACTTACATTGATCCACATACCTATGAGG
ATCCCAATCAAGCTGTCCACGAATTTGCTAAGGAGATAGAAGCATCATG
TATCACCATTGAGAGAGTTATTGGAGCAGGTGAATTTGGTGAAGTTTGT
AGTGGACGTTTGAAACTACCAGGAAAAAGAGAATTACCTGTGGCTATCA
AAACCCTTAAAGTAGGCTATACTGAAAAGCAACGCAGAGATTTCCTAGG
TGAAGCAAGTATCATGGGACAGTTTGATCATCCTAACATCATCCATTTA
GAAGGTGTGGTGACCAAAAGTAAACCAGTGATGATCGTGACAGAGTATA
TGGAGAATGGCTCTTTAGATACATTTTTGAAGAAAAACGATGGGCAGTT
CACTGTGATTCAGCTTGTTGGCATGCTGAGAGGTATCTCTGCAGGAATG
AAGTACCTTTCTGACATGGGCTATGTGCATAGAGATCTTGCTGCCAGAA
ACATCTTAATCAACAGTAACCTTGTGTGCAAAGTGTCTGACTTTGGACT
TTCCCGGGTACTGGAAGATGATCCCGAGGCAGCCTACACCACAAGGGGA
GGAAAAATTCCAATCAGATGGACTGCCCCAGAAGCAATAGCTTTCCGAA
AGTTTACTTCTGCCAGTGATGTCTGGAGTTATGGAATAGTAATGTGGGA
AGTTGTGTCTTATGGAGAGAGACCCTACTGGGAGATGACCAATCAAGAT
GTGATTAAAGCGGTAGAGGAAGGCTATCGTCTGCCAAGCCCCATGGATT
GTCCTGCTGCTCTCTATCAGTTAATGCTGGATTGCTGGCAGAAAGAGCG
AAATAGCAGGCCCAAGTTTGATGAAATAGTCAACATGTTGGACAAGCTG
ATACGTAACCCAAGTAGTCTGAAGACGCTGGTTAATGCATCCTGCAGAG
TATCTAATTTATTGGCAGAACATAGCCCACTAGGATCTGGGGCCTACAG
ATCAGTAGGTGAATGGCTAGAGGCAATCAAGATGGGCCGGTATACAGAG
ATTTTCATGGAAAATGGATACAGTTCAATGGACGCTGTGGCTCAGGTGA
CCTTGGAGGATTTGAGACGGCTTGGAGTGACTCTTGTCGGTCACCAGAA
GAAGATCATGAACAGCCTTCAAGAAATGAAGGTGCAGCTGGTAAACGGA
ATGGTGCCATTGtaagcggccgc