Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active EPHA7 [579 – 998]

Enzyme description:- EPH A7 [579 - 998]

Clone number:- DU 63369

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 74, 153.40 daltons
Average Mass 74, 201.66 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.14

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:-
Poly Glu Tyr (4:1) Final concentration: 0.1 mg/ml

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Clone Data Sheet

EPHA7 [579 - 998]

Protein	EPH A7 [579 - 998]
Clone number	DU 63369
Species	Human
Accession number	NM_004440.3
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**GRRHCGYSKADQEG DEELYFHFKFPGTKTYIDPETYEDPNRAVHQFAKELDASCIKIERVIGA GEFGEVCSGRLKLPGKRDVAVAIKTLKVGYTEKQRRDFLCEASIMGQFD HPNVVHLEGVVTRGKPVMIVIEFMENGALDAFLRKHDGQFTVIQLVGML RGIAAGMRYLADMGYVHRDLAARNILVNSNLVCKVSDFGLSRVIEDDPE AVYTTTGGKIPVRWTAPEAIQYRKFTSASDVWSYGIVMWEVMSYGERPY WDMSNQDVIKAIEEGYRLPAPMDCPAGLHQLMLDCWQKERAERPKFEQI VGILDKMIRNPNSLKTPLGTCSRPISPLLDQNTPDFTTFCSVGEWLQAI KMERYKDNFTAAGYNSLESVARMTIEDVMSLGITLVGHQKKIMSSIQTM RAQMLHLHGTGIQV**
Native sequence	Amino acids G579 – V998 (end) of human EPH A7. Residue G232 of the fusion protein is equivalent to G579 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGPL) residues 221 - 229
Cloning sites	*Bam*H1 and *Not*1 site of pGEX 6P-1

Nucleotide Sequence Of Insert

ggatccgGGAGAAGGCACTGTGGTTATAGCAAAGCTGACCAAGAAGGCG
ATGAAGAGCTTTACTTTCATTTTAAATTTCCAGGCACCAAAACCTACAT
TGACCCTGAAACCTATGAGGACCCAAATAGAGCTGTCCATCAATTCGCC
AAGGAGCTAGATGCCTCCTGTATTAAAATTGAGCGTGTGATTGGTGCAG
GAGAATTCGGTGAAGTCTGCAGTGGCCGTTTGAAACTTCCAGGGAAAAG
AGATGTTGCAGTAGCCATAAAAACCCTGAAAGTTGGTTACACAGAAAAA
CAAAGGAGAGACTTTTTGTGTGAAGCAAGCATCATGGGGCAGTTTGACC
ACCCGAATGTTGTCCATTTGGAAGGGGTTGTTACAAGAGGGAAACCAGT
CATGATAGTAATAGAGTTCATGGAAAATGGAGCCCTAGATGCATTTCTC
AGGAAACATGATGGGCAATTTACAGTCATTCAGTTAGTAGGAATGCTGA
GAGGAATTGCTGCTGGAATGAGATATTTGGCTGATATGGGATATGTTCA
CAGGGACCTTGCAGCTCGCAATATTCTTGTCAACAGCAATCTCGTTTGT
AAAGTGTCAGATTTTGGCCTGTCCCGAGTTATAGAGGATGATCCAGAAG
CTGTCTATACAACTACTGGTGGAAAAATTCCAGTAAGGTGGACAGCACC
CGAAGCCATCCAGTACCGGAAATTCACATCAGCCAGTGATGTATGGAGC
TATGGAATAGTCATGTGGGAAGTTATGTCTTATGGAGAAAGACCTTATT
GGGACATGTCAAATCAAGATGTTATAAAAGCAATAGAAGAAGGTTATCG
TTTACCAGCACCCATGGACTGCCCAGCTGGCCTTCACCAGCTAATGTTG
GATTGTTGGCAAAAGGAGCGTGCTGAAAGGCCAAAATTTGAACAGATAG
TTGGAATTCTAGACAAAATGATTCGAAACCCAAATAGTCTGAAAACTCC
CCTGGGAACTTGTAGTAGGCCAATAAGCCCTCTTCTGGATCAAAACACT
CCTGATTTCACTACCTTTTGTTCAGTTGGAGAATGGCTACAAGCTATTA
AGATGGAAAGATATAAAGATAATTTCACGGCAGCTGGCTACAATTCCCT
TGAATCAGTAGCCAGGATGACTATTGAGGATGTGATGAGTTTAGGGATC
ACACTGGTTGGTCATCAAAAGAAAATCATGAGCAGCATTCAGACTATGA
GAGCACAAATGCTACATTTACATGGAACTGGCATTCAAGTGtgagcggc
cgc