Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active EPHA8 [565 – 1005]

Enzyme description:- EPH A8 [565 - 1005]

Clone number:- DU 63351

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 76, 285.52 daltons
Average Mass 76, 334.65 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 7.31

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

EPHA8 [565 - 1005]

Protein	EPH A8 [565 - 1005]
Clone number	DU 63351
Species	Human
Accession number	NM_020526.4
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEF**KRHCGYSKAFQ** **DSDEEKMHYQNGQAPPPVFLPLHHPPGKLPEPQFYAEPHTYEEPGRAGR** **SFTREIEASRIHIEKIIGSGDSGEVCYGRLRVPGQRDVPVAIKALKAGY** **TERQRRDFLSEASIMGQFDHPNIIRLEGVVTRGRLAMIVTEYMENGSLD** **TFLRTHDGQFTIMQLVGMLRGVGAGMRYLSDLGYVHRDLAARNVLVDSN** **LVCKVSDFGLSRVLEDDPDAAYTTTGGKIPIRWTAPEAIAFRTFSSASD** **VWSFGVVMWEVLAYGERPYWNMTNRDVISSVEEGYRLPAPMGCPHALHQ** **LMLDCWHKDRAQRPRFSQIVSVLDALIRSPESLRATATVSRCPPPAFVR** **SCFDLRGGSGGGGGLTVGDWLDSIRMGRYRDHFAAGGYSSLGMVLRMNA** **QDVRALGITLMGHQKKILGSIQTMRAQLTSTQGPRRHL**
Native sequence	Amino acids K565 – L1005 (end) of human EPH A8. Residue K235 of the fusion protein is equivalent to K565 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

gaattcAAGAGGCACTGTGGCTACAGCAAGGCCTTCCAGGACTCGGACG
AGGAGAAGATGCACTATCAGAATGGACAGGCACCCCCACCTGTCTTCCT
GCCTCTGCATCACCCCCCGGGAAAGCTCCCAGAGCCCCAGTTCTATGCG
GAACCCCACACCTACGAGGAGCCAGGCCGGGCGGGCCGCAGTTTCACTC
GGGAGATCGAGGCCTCTAGGATCCACATCGAGAAAATCATCGGCTCTGG
AGACTCCGGGGAAGTCTGCTACGGGAGGCTGCGGGTGCCAGGGCAGCGG
GATGTGCCCGTGGCCATCAAGGCCCTCAAAGCCGGCTACACGGAGAGAC
AGAGGCGGGACTTCCTGAGCGAGGCGTCCATCATGGGGCAATTCGACCA
TCCCAACATCATCCGCCTCGAGGGTGTCGTCACCCGTGGCCGCCTGGCA
ATGATTGTGACTGAGTACATGGAGAACGGCTCTCTGGACACCTTCCTGA
GGACCCACGACGGGCAGTTCACCATCATGCAGCTGGTGGGCATGCTGAG
AGGAGTGGGTGCCGGCATGCGCTACCTCTCAGACCTGGGCTATGTCCAC
CGAGACCTGGCCGCCCGCAACGTCCTGGTTGACAGCAACCTGGTCTGCA
AGGTGTCTGACTTCGGGCTCTCACGGGTGCTGGAGGACGACCCGGATGC
TGCCTACACCACCACGGGCGGGAAGATCCCCATCCGCTGGACGGCCCCA
GAGGCCATCGCCTTCCGCACCTTCTCCTCGGCCAGCGACGTGTGGAGCT
TCGGCGTGGTCATGTGGGAGGTGCTGGCCTATGGGGAGCGGCCCTACTG
GAACATGACCAACCGGGATGTCATCAGCTCTGTGGAGGAGGGGTACCGC
CTGCCCGCACCCATGGGCTGCCCCCACGCCCTGCACCAGCTCATGCTCG
ACTGTTGGCACAAGGACCGGGCGCAGCGGCCTCGCTTCTCCCAGATTGT
CAGTGTCCTCGATGCGCTCATCCGCAGCCCTGAGAGTCTCAGGGCCACC
GCCACAGTCAGCAGGTGCCCACCCCCTGCCTTCGTCCGGAGCTGCTTTG
ACCTCCGAGGGGGCAGCGGTGGCGGTGGGGGCCTCACCGTGGGGGACTG
GCTGGACTCCATCCGCATGGGCCGGTACCGAGACCACTTCGCTGCGGGC
GGATACTCCTCTCTGGGCATGGTGCTACGCATGAACGCCCAGGACGTGC
GCGCCCTGGGCATCACCCTCATGGGCCACCAGAAGAAGATCCTGGGCAG
CATTCAGACCATGCGGGCCCAGCTGACCAGCACCCAGGGGCCCCGCCGG
CACCTCtgagcggccgc