Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active EPHA3 [568 – 983]

Enzyme description:- EPH A3 [568 - 983]

Clone number:- DU 63469

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 73, 236.39 daltons
Average Mass 73, 283.43 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.75

Purity:- 80 %

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: 1 mg/ml

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Clone Data Sheet

EPHA3 [568 - 983]

Protein EPH A1 [568 - 983]

Clone number DU 63469

Species Human

Accession number NM_005233.6

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**CGYKSKHGADEKRL**
HFGNGHLKLPGLRTYVDPHTYEDPTQAVHEFAKELDATNISIDKVVGAG
EFGEVCSGRLKLPSKKEISVAIKTLKVGYTEKQRRDFLGEASIMGQFDH
PNIIRLEGVVTKSKPVMIVTEYMENGSLDSFLRKHDAQFTVIQLVGMLR
GIASGMKYLSDMGYVHRDLAARNILINSNLVCKVSDFGLSRVLEDDPEA
AYTTRGGKIPIRWTSPEAIAYRKFTSASDVWSYGIVLWEVMSYGERPYW
EMSNQDVIKAVDEGYRLPPPMDCPAALYQQMLDCWQKDRNNRPKFEQIV
SILDKLIRNPGSLKIITSAAARPSNLLLDQSNVDITTFRTTGDWLNGVW
TAHCKEIFTGVEYSSCDTIAKISTDDMKKVGVTVVGPQKKIISSIKALE
TQSKNGPVPV

Native sequence Amino acids C568 – V983 (end) of human EPH A3.
Residue 232C of the fusion protein is equivalent to C568 of the native enzyme. The GST tag is located at residues 1 – 220.

The enzyme has a L856Q mutation. Residues L856 is equivalent to Q520 of the fusion protein

Protease cleavage PreScission (LEVLFQGPL) residues 221 - 229

Cloning sites *Bam*H1 and *Not*1 site of pGEX 6P-1

Nucleotide Sequence Of Insert

ggatcctGTGGCTATAAGTCAAAACATGGGGCAGATGAAAAAAGACTTC
ATTTTGGCAATGGGCATTTAAAACTTCCAGGTCTCAGGACTTATGTTGA
CCCACATACATATGAAGACCCTACCCAAGCTGTTCATGAGTTTGCCAAG
GAATTGGATGCCACCAACATATCCATTGATAAAGTTGTTGGAGCAGGTG
AATTTGGAGAGGTGTGCAGTGGTCGCTTAAAACTTCCTTCAAAAAAAGA
GATTTCAGTGGCCATTAAGACCCTGAAAGTTGGCTACACAGAAAAGCAG
AGGAGAGACTTCCTGGGAGAAGCAAGCATTATGGGACAGTTTGACCACC
CCAATATCATTCGACTGGAAGGAGTTGTTACCAAAAGTAAGCCAGTTAT
GATTGTCACAGAATACATGGAGAATGGTTCCTTGGATAGTTTCCTACGT
AAACACGATGCCCAGTTTACTGTCATTCAGCTAGTGGGGATGCTTCGAG
GGATAGCATCTGGCATGAAGTACCTGTCAGACATGGGCTATGTTCACCG
AGACCTCGCTGCTCGGAACATCTTGATCAACAGTAACTTGGTGTGTAAG
GTTTCTGATTTCGGACTTTCGCGTGTCCTGGAGGATGACCCAGAAGCTG
CTTATACAACAAGAGGAGGGAAGATCCCAATCAGGTGGACATCACCAGA
AGCTATAGCCTACCGCAAGTTCACGTCAGCCAGCGATGTATGGAGTTAT
GGGATTGTTCTCTGGGAGGTGATGTCTTATGGAGAGAGACCATACTGGG
AGATGTCCAATCAGGATGTAATTAAAGCTGTAGATGAGGGCTATCGACT
GCCACCCCCCATGGACTGCCCAGCTGCCTTGTATCAGCAGATGCTGGAC
TGCTGGCAGAAAGACAGGAACAACAGACCCAAGTTTGAGCAGATTGTTA
GTATTCTGGACAAGCTTATCCGGAATCCCGGCAGCCTGAAGATCATCAC
CAGTGCAGCCGCAAGGCCATCAAACCTTCTTCTGGACCAAAGCAATGTG
GATATCACTACCTTCCGCACAACAGGTGACTGGCTTAATGGTGTCTGGA
CAGCACACTGCAAGGAAATCTTCACGGGTGTGGAGTACAGTTCTTGTGA
CACAATAGCCAAGATTTCCACAGATGACATGAAAAAGGTTGGTGTCACC
GTGGTTGGGCCACAGAAGAAGATCATCAGTAGCATTAAAGCTCTAGAAA
CGCAATCAAAGAATGGCCCAGTTCCCGTGtaagcggccgc