Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active EPHA2 [591 – 976] D757A

Enzyme description:- EPH A2 [591 - 976] D757A

Clone number:- DU 53693

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 70, 310.93 daltons
Average Mass 70, 356.52 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 7.21

Purity:- 90 %

Activation protocol:- Kinase dead

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

EPHA2 [591 - 976] D757A

Protein	EPH A2 [591 - 976] D757A
Clone number	DU 4438
Species	Human
Accession number	NM_004431
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**PHTYEDPNQAVLKF** **TTEIHPSCVTRQKVIGAGEFGEVYKGMLKTSSGKKEVPVAIKTLKAGYT** **EKQRVDFLGEAGIMGQFSHHNIIRLEGVISKYKPMMIITEYMENGALDK** **FLREKDGEFSVLQLVGMLRGIAAGMKYLANMNYVHRDLAARNILVNSNL** **VCKVSAFGLSRVLEDDPEATYTTSGGKIPIRWTAPEAISYRKFTSASDV** **WSFGIVMWEVMTYGERPYWELSNHEVMKAINDGFRLPTPMDCPSAIYQL** **MMQCWQQERARRPKFADIVSILDKLIRAPDSLKTLADFDPRVSIRLPST** **SGSEGVPFRTVSEWLESIKMQQYTEHFMAAGYTAIEKVVQMTNDDIKRI** **GVRLPGHQKRIAYSLLGLKDQVNTVGIPI**
Native sequence	Amino acids P591 – I976 (end) of human EPH A2. Residue P232 of the fusion protein is equivalent to P591 of the native enzyme. The GST tag is located at residues 1 – 220. The enzyme has a D757A mutation, which produce a kinase dead enzyme. Residue D757 is equivalent to A398 of the fusion protein.
Protease cleavage	PreScission (LEVLFQGPL) residues 221 - 229
Cloning sites	*Bgl*III and *Sal*1site of pGEX 6P-1

Nucleotide Sequence

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCA
CTCGACTTCTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTA
TGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGT
TTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAA
CACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAACATGTT
GGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCG
GTTTTGGATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACT
TTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAA
AATGTTCGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCAT
GTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTAT
ACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAA
AAAACGTATTGAAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGC
AAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTG
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GGGATCTCCCCACACATATGAGGACCCCAACCAGGCTGTGTTGAAGTTC
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GCCCATGATGATCATCACTGAGTACATGGAGAATGGGGCCCTGGACAAG
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GACCCTGGCTGACTTTGACCCCCGCGTGTCTATCCGGCTCCCCAGCACG
AGCGGCTCGGAGGGGGTGCCCTTCCGCACGGTGTCCGAGTGGCTGGAGT
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TGCCATCGAGAAGGTGGTGCAGATGACCAACGACGACATCAAGAGGATT
GGGGTGCGGCTGCCCGGCCACCAGAAGCGCATCGCCTACAGCCTGCTGG
GACTCAAGGACCAGGTGAACACTGTGGGGATCCCCATCtga