University of Dundee

Standard Operating Procedure

Preparation of active EPH A2 [591 – 976]

Enzyme description:- EPH A2 [591 - 976]

Clone number:- DU 4438

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 6 mg/L

Calculated molecular mass:-
Monoisotopic 70, 354.92 daltons
Average Mass 70, 400.53 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.94

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

Specific activity range:- To be determined

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Clone Data Sheet

EPH A2 [591 - 976]

Protein	EPH A2 [591 - 976]
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** **VCKVSDFGLSRVLEDDPEATYTTSGGKIPIRWTAPEAISYRKFTSASDV** **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.
Protease cleavage	PreScission (LEVLFQGPL) residues 221 - 229
Cloning sites	*Bgl*III and *Sal*1site of pGEX 6P-1

Nucleotide Sequence

atgtcccctatactaggttattggaaaattaagggccttgtgcaaccca
ctcgacttcttttggaatatcttgaagaaaaatatgaagagcatttgta
tgagcgcgatgaaggtgataaatggcgaaacaaaaagtttgaattgggt
ttggagtttcccaatcttccttattatattgatggtgatgttaaattaa
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GACCCTGGCTGACTTTGACCCCCGCGTGTCTATCCGGCTCCCCAGCACG
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TGCCATCGAGAAGGTGGTGCAGATGACCAACGACGACATCAAGAGGATT
GGGGTGCGGCTGCCCGGCCACCAGAAGCGCATCGCCTACAGCCTGCTGG
GACTCAAGGACCAGGTGAACACTGTGGGGATCCCCATCtgagtcgac