Certificate of Analysis

ProQinase TARGETING CANCER

Recombinant Proteins

EPHA2

EPH receptor A2

Recombinant Human Active Protein Kinase

HGNC Symbol: EPHA2

Synonyms: ARCC2, ECK

Product No.: 0368-0000-1

Lot: 001

Description: Human EPHA2, C-terminal fragment, amino acids L_{585}-I_{976} (as in NCBI/Protein entry NP_004422.2), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: EPHA2 Lot 001, was confirmed as EPHA2 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 73,691 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

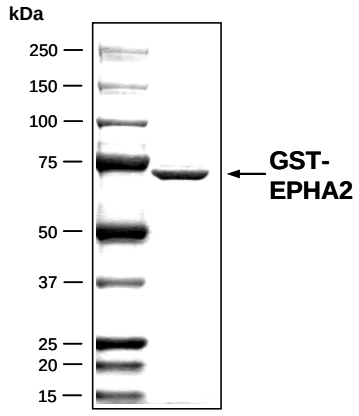
Storage buffer: 50 mM TRIS-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20 % glycerol

Storage temperature: -80°C
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.505 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

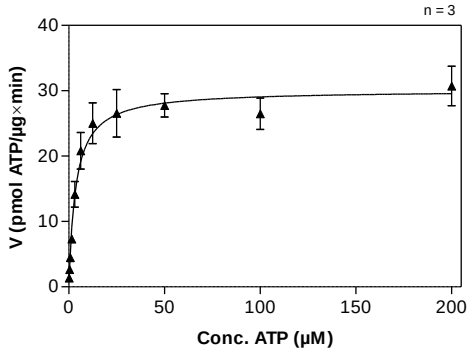
Biochemical Parameters:
Specific kinase activity (P_i transfer): 30 pmol/µg × min
ATP-K_M: 3.5 µM

EPHA2 Lot 001:
Coomassie stain



2.0 µg GST-EPHA2

EPHA2 Lot 001:
Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:
- Assay conditions:
  60 mM HEPES-NaOH, pH 7.5
  3 mM $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg/ml $PEG_{20.000}$
  ATP (variable)
  Substrate: $Poly(Glu/Tyr)_{4:1}$ 40 µg/ml
  Kinase: 2 µg/ml
- Filter binding assay

MSFC membrane (Millipore)

Additional assay technology:
EPHA2 Lot 001 was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega
ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

ProQinase
TARGETING CANCER

EPHA2

Product No.: 0368-0000-1

GST-EPHA2 Recombinant Fusion Protein Amino Acid Sequence

```
  1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID  60
 61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV 120
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RRRASVAAGI 240
241 LVPRGSPGLD GICSRLKTYV DPHTYEDPNQ AVLKFTTEIH PSCVTRQKVI GAGEFGEVYK 300
301 GMLKTSSGKK EVPVAIKTLK AGYTEKQRVD FLGEAGIMGQ FSHHNIIRLE GVISKYKPMM 360
361 IITEYMENGA LDKFLREKDG EFSVLQLVGM LRGIAAGMKY LANMNYVHRD LAARNILVNS 420
421 NLVCKVSDFG LSRVLEDDPE ATYTTSGGKI PIRWTAPEAI SYRKFTSASD VWSFGIVMWE 480
481 VMTYGERPYW ELSNHEVMKA INDGFRLPTP MDCPSAIYQL MMQCWQQERA RRPKFADIVS 540
541 ILDKLIRAPD SLKTLADFDP RVSIRLPSTS GSEGVPFRTV SEWLESIKMQ QYTEHFMAAG 600
601 YTAIEKVVQM TNDDIKRIGV RLPGHQKRIA YSLLGLKDQV NTVGIPI              660
```

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: EPHA2 fragment

EPHA2 wt[1] Amino Acid Sequence

```
  1 MELQAARACF ALLWGCALAA AAAAQGKEVV LLDFAAAGGE LGWLTHPYGK GWDLMQNIMN  60
 61 DMPIYMYSVC NVMSGDQDNW LRTNWVYRGE AERIFIELKF TVRDCNSFPG GASSCKETFN 120
121 LYYAESDLDY GTNFQKRLFT KIDTIAPDEI TVSSDFEARH VKLNVEERSV GPLTRKGFYL 180
181 AFQDIGACVA LLSVRVYYKK CPELLQGLAH FPETIAGSDA PSLATVAGTC VDHAVVPPGG 240
241 EEPRMHCAVD GEWLVPIGQC LCQAGYEKVE DACQACSPGF FKFEASESPC LECPEHTLPS 300
301 PEGATSCECE EGFFRAPQDP ASMPCTRPPS APHYLTAVGM GAKVELRWTP PQDSGGREDI 360
361 VYSVTCEQCW PESGECGPCE ASVRYSEPPH GLTRTSVTVS DLEPHMNYTF TVEARNGVSG 420
421 LVTSRSFRTA SVSINQTEPP KVRLEGRSTT SLSVSWSIPP PQQSRVWKYE VTYRKKGDSN 480
481 SYNVRRTEGF SVTLDDLAPD TTYLVQVQAL TQEGQGAGSK VHEFQTLSPE GSGNLAVIGG 540
541 VAVGVVLLLV LAGVGFFIHR RRKNQRARQS PEDVYFSKSE QLKPLKTYVD PHTYEDPNQA 600
601 VLKFTTEIHP SCVTRQKVIG AGEFGEVYKG MLKTSSGKKE VPVAIKTLKA GYTEKQRVDF 660
661 LGEAGIMGQF SHHNIIRLEG VISKYKPMMI ITEYMENGAL DKFLREKDGE FSVLQLVGML 720
721 RGIAAGMKYL ANMNYVHRDL AARNILVNSN LVCKVSDFGL SRVLEDDPEA TYTTSGGKIP 780
781 IRWTAPEAIS YRKFTSASDV WSFGIVMWEV MTYGERPYWE LSNHEVMKAI NDGFRLPTPM 840
841 DCPSAIYQLM MQCWQQERAR RPKFADIVSI LDKLIRAPDS LKTLADFDPR VSIRLPSTSG 900
901 SEGVPFRTVS EWLESIKMQQ YTEHFMAAGY TAIEKVVQMT NDDIKRIGVR LPGHQKRIAY 960
961 SLLGLKDQVN TVGIPI                                              1020
```

blue: EPHA2 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_004422.2

Recombinant Proteins