# Certificate of Analysis

REACTION BIOLOGY

# ProQinase™ EPHB3

**EPH receptor B3**

## Recombinant Human Active Protein Kinase

**HGNC Symbol:** EPHB3

**Synonyms:** Hek2, Tyro6, ETK2

**Product No.:** 0231-0000-1

**Lot:** 001

**Description:** Human Kinase, C-terminal fragment, amino acids $Q_{585}$-$V_{998}$ (as in NCBI/Protein entry NP_004434.1), N-terminal GST-$HIS_6$ fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

**Product identity:** EPHB3 Lot 001, has been verified by mass spectrometry LC-ESI-MS/MS

**Theoretical $MW_{Fusion\ Protein}$:** 80148 Da

**Expression host:** Sf9 insect cells

**Purification:** GST-Affinity Chromatography

**Activation:** This kinase was not activated by special procedures

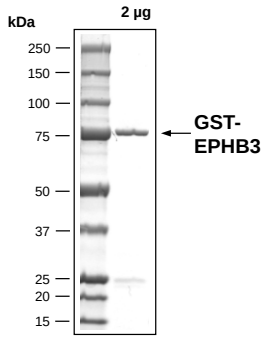
**Storage buffer:** 50 mM TRIS-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

**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!

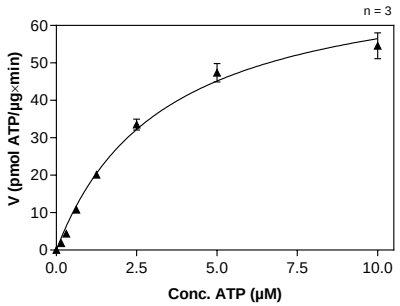
**Protein concentration:** 0.258 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

**Biochemical Parameters:**
Specific kinase activity ($P_i$ transfer): 75 pmol/µg*min
ATP-$K_M$: 3.35 µM

**EPHB3 Lot 001:**
**Coomassie stain**




**EPHB3 Lot 001:**
**Determination of $V_{max}$ and $K_M$ value for ATP**




- 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: $Poly(E/Y)_{4:1}$ 10 µg/ml
  Kinase: 0.5 µg/ml

Assay technology:
Radiometric filter binding assay
MSFC membrane (96 well plate, Millipore)

Recombinant Proteins

## Sequence information

**GST-EPHB3 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 GIYARGIQAS MGARGRQCDG YLQNSPLCMQ RHGSDSEYTE KLQQYIAPGM 300
301 KVYIDPFTYE DPNEAVREFA KEVDVSCVKI EEVIGAGEFG EVCRGRLKQP GRREVFVAIK 360
361 TLKVGYTERQ RRDFLSEASI MGQFDHPNII RLEGVVTKSR PVMILTEFME NCALDSFLRL 420
421 NDGQFTVIQL VGMLRGIAAG MKYLSEMNYV HRDLAARNIL VNSNLVCKVS DFGLSRFLED 480
481 DPSDPTYTSS LGGKIPIRWT APEAIAYRKF TSASDVWSYG IVMWEVMSYG ERPYWDMSNQ 540
541 DVINAVEQDY RLPPPMDCPT ALHQLMLDCW VRDRNLRPKF SQIVNTLDKL IRNAASLKVI 600
601 ASAQSGMSQP LLDRTVPDYT TFTTVGDWLD AIKMGRYKES FVSAGFASFD LVAQMTAEDL 660
661 LRIGVTLAGH QKKILSSIQD MRLQMNQTLP VQVKGEFQHT GGRY                   720
```

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: EPHB3 fragment **boxed**: variation from RefSeq

**EPHB3 wt[1] Amino Acid Sequence**

```
  1 MARARPPPPP SPPPGLLPLL PPLLLLPLLL LPAGCRALEE TLMDTKWVTS ELAWTSHPES  60
 61 GWEEVSGYDE AMNPIRTYQV CNVRESSQNN WLRTGFIWRR DVQRVYVELK FTVRDCNSIP 120
121 NIPGSCKETF NLFYYEADSD VASASSPFWM ENPYVKVDTI APDESFSRLD AGRVNTKVRS 180
181 FGPLSKAGFY LAFQDQGACM SLISVRAFYK KCASTTAGFA LFPETLTGAE PTSLVIAPGT 240
241 CIPNAVEVSV PLKLYCNGDG EWMVPVGACT CATGHEPAAK ESQCRPCPPG SYKAKQGEGP 300
301 CLPCPPNSRT TSPAASICTC HNNFYRADSD SADSACTTVP SPPRGVISNV NETSLILEWS 360
361 EPRDLGGRDD LLYNVICKKC HGAGGASACS RCDDNVEFVP RQLGLTERRV HISHLLAHTR 420
421 YTFEVQAVNG VSGKSPLPPR YAAVNITTNQ AAPSEVPTLR LHSSSGSSLT LSWAPPERPN 480
481 GVILDYEMKY FEKSEGIAST VTSQMNSVQL DGLRPDARYV VQVRARTVAG YGQYSRPAEF 540
541 ETTSERGSGA QQLQEQLPLI VGSATAGLVF VVAVVVIAIV CLRKQRHGSD SEYTEKLQQY 600
601 IAPGMKVYID PFTYEDPNEA VREFAKEIDV SCVKIEEVIG AGEFGEVCRG RLKQPGRREV 660
661 FVAIKTLKVG YTERQRRDFL SEASIMGQFD HPNIIRLEGV VTKSRPVMIL TEFMENCALD 720
721 SFLRLNDGQF TVIQLVGMLR GIAAGMKYLS EMNYVHRDLA ARNILVNSNL VCKVSDFGLS 780
781 RFLEDDPSDP TYTSSLGGKI PIRWTAPEAI AYRKFTSASD VWSYGIVMWE VMSYGERPYW 840
841 DMSNQDVINA VEQDYRLPPP MDCPTALHQL MLDCWVRDRN LRPKFSQIVN TLDKLIRNAA 900
901 SLKVIASAQS GMSQPLLDRT VPDYTTFTTV GDWLDAIKMG RYKESFVSAG FASFDLVAQM 960
961 TAEDLLRIGV TLAGHQKKIL SSIQDMRLQM NQTLPVQV                        1020
```

**blue**: EPHB3 sequence expressed in recombinant protein **Red**: variant in recombinant protein

[1]NCBI/Protein accession number NP_004434.1

Recombinant Proteins