

# Certificate of Analysis



## EPHA3

Eph receptor A3

### Recombinant Human Active Protein Kinase

**HGNC Symbol:** EPHA3

**Synonyms:** ETK; ETK1; EphA3; HEK; HEK4; TYRO4; EPH receptor A3

**Product No.:** 0351-0000-1

**Lot:** 001

**Description:** Human EPHA3, C-terminal fragment, amino acids G<sub>569</sub>-V<sub>983</sub> (as in NCBI/Protein entry NP\_005224.2), N-terminal GST-HIS<sub>6</sub> fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

**Product identity:** EPHA3 Lot 001, was confirmed as EPHA3 by mass spectroscopy LC-ESI-MS/MS

**Theoretical MW<sub>Fusion Protein</sub>:** 75,680 Da

**Expression:** Baculovirus infected Sf9 cells

**Purification:** GST-Affinity Chromatography

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

**Storage buffer:** 50 mM HEPES pH 7.5, 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:** 0.140 µg/µl

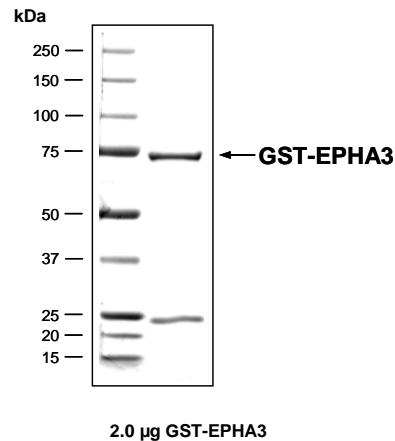
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

### Biochemical Parameters:

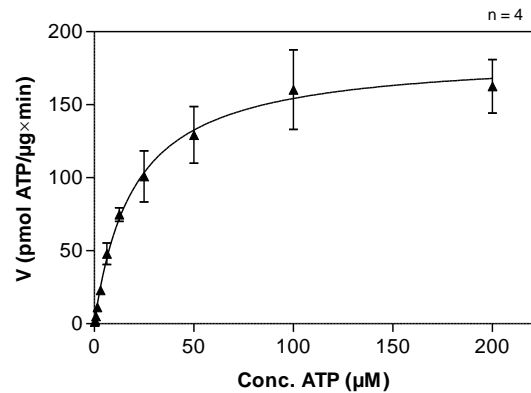
Specific kinase activity (P<sub>i</sub> transfer): 184 pmol/µg×min

ATP-K<sub>M</sub>: 19.6 µM

### EPHA3 Lot 001: Coomassie stain



### EPHA3 Lot 001: Determination of V<sub>max</sub> and K<sub>M</sub> value for ATP



### Determination of K<sub>M</sub> value & Specific activity:

- Assay conditions:
  - 60 mM HEPES-NaOH, pH 7.5
  - 3 mM MgCl<sub>2</sub>
  - 3 mM MnCl<sub>2</sub>
  - 3 µM Na-orthovanadate
  - 1.2 mM DTT
  - 50 µg / ml PEG<sub>20,000</sub>
  - ATP (variable)
  - Substrate: Poly(Glu:Tyr)<sub>4:1</sub>, 20 µg/ml
  - EPHA3: 4.0 µg/ml
- Filter binding assay
  - MSFC membrane (Millipore)

### Additional assay technology: EPHA3 Lot 001

was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega



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# EPHA3

Product No.: 0351-0000-1

EPHA3 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQDMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHGG	RRRASVAAGI	240
241	LVPRGSPGLD	GICSRGYKSK	HGADEKRLHF	GNGHLKLPGL	RTYVDPHTYE	DPTQAVHEFA	300
301	KELDATNISI	DKVVGAGEFG	EVCSGRLKLP	SKKEISVAIK	TLKVGYTEKQ	RRDFLGEASI	360
361	MGQFDHPNII	RLEGVVTKSK	PVMIVTEYME	NGSLDSFLRK	HDAQFTVIQL	VGMLRGIASG	420
421	MKYLSDMGYV	HRDLAARNIL	INSNLVCKVS	DFGLSRVLED	DPEAAYTTRG	GKIPIRWTSP	480
481	EAIAYRKFTS	ASDVWSYGIV	LWEVMSYGER	PYWEMSNDQV	IKAVDEGYRL	PPMDCPAAS	540
541	YQLMLDCWQK	DRNNRPKFEQ	IVSILDKLIR	NPGSLKIITS	AAARPSNLLL	DQSNVDITTF	600
601	RTTGDWLNQV	RTAHCKEIFT	GVEYSSCDTI	AKISTDDMKK	VGVTVVGPOK	KIISSEKALE	660
661	TQSKNGPVPV						720

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: EPHA3 fragment **boxed**: variation from RefSeq

EPHA3 wt <sup>1</sup> amino acid sequence							
1	MDCQLSILLL	LSCSVLDSFG	ELIQPSNEV	NLLDSKTIQG	ELGWISYPSH	GWEEISGVDE	60
61	HYTPIRTYQV	CNVMDHSQNN	WLRTNWWVRN	SAQKIYVELK	FTLRDCNSIP	LVLGTCKETF	120
121	NLYMESDDD	HGVKFRHQF	TKIDTIAADE	SFTQMDLGDR	ILKLNTEIRE	VGPVNKKGFY	180
181	LAFQDVGACV	ALVSVRVYFK	KCPFTVKNLA	MFPDTPMDS	QSLVEVRGSC	VNNSKEEDPP	240
241	RMYCSTEGEW	LVPIGKCSN	AGYEERGFMC	QACRPGFYKA	LDGNMKCAKC	PPHSSTQEDG	300
301	SMNCRCENNY	FRADKDPPSM	ACTRPPSSPR	NVISNINETS	VILDWSWPLD	TGGRKDVTFN	360
361	IICKKCGWNI	KQCEPCSPNV	RFLPRQFGLT	NTTIVTVDDL	AHTNYTFEID	AVNGVSELSS	420
421	PPRQFAAVSI	TTNQAAPSPV	LTIKKDRTSR	NSISLSWQEP	EHPNGIILDY	EVKYYEKQEQ	480
481	ETSYTILRAR	GTNVTISSLK	PDTIYVFQIR	ARTAAGYGTN	SRKFEFETSP	DSFISISGESS	540
541	QVVMIAISAA	VAIILLTVVI	YVLIGRFCGY	KSKHGADEKR	LHFGNGHLKL	PGLRTYVDPH	600
601	TYEDPTQAVH	EFAKELDATN	ISIDKVVGAG	EFGEVCSGRL	KLPSKKEISV	AIKTLKVGYT	660
661	EKQRRDFLGE	ASIMGQFDHP	NIIRLEGVVT	KSKPVMIVTE	YMENGLDSF	LRKHDAQFTV	720
721	IQLVGMLRGI	ASGMKYLSDM	GYVHRDLAAR	NILINSNLVC	KVSDFGLSRV	LEDDPEAAYT	780
781	TRGGKIPIRW	TSPEAIAYRK	FTSASDVWSY	GIVLWEVMSY	GERPYWEMSN	QDVIKAVDEG	840
841	YRLPPPMDCP	AALYQLMLDC	WQKDRNNRPK	FEQIVSILDK	LIRNPGSLKI	ITSAAARPSN	900
901	LLLDQSNVDI	TTFRITGDWL	NGVWTAHCKE	IFTGVEYSSC	DTIAKISTDD	MKKVGVTVVG	960
961	PQKKIISSEK	ALETQSKNGP	VPV				1020

**blue**: EPHA3 sequence expressed in fusionprotein **Red**: variant in fusionprotein

<sup>1</sup>NCBI/Protein accession number NP\_005224.2  
W924R: SNP variation see NCBI/dbSNP ID: rs35124509