

Certificate of Analysis

FGF-R4

fibroblast growth factor receptor 4

Recombinant Human Active Protein Kinase

HGNC Symbol: FGFR4

Synonyms: CD334, JTK, TKF

Product No.: 0127-0000-3

Lot: 006

Description: Human FGF-R4 Amino acids R₃₉₁-T₈₀₂ (as in NCBI/Protein entry NP_002002.3), N-terminally fused to GST-HIS₆-Thrombin cleavage site

Product identity: FGF-R4, Lot 007, was confirmed as FGF-R4 by specific Western Blotting using anti FGF-R4 antibody

Theoretical MW_{Fusion Protein}: 75,726 Da

Expression: Baculovirus infected Sf9 cells

Purification: One-step affinity purification using GSH-agarose

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
Avoid repeated freeze-thaw cycles!

Protein concentration: 0.245 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Method for determination of K_m value & Specific activity:

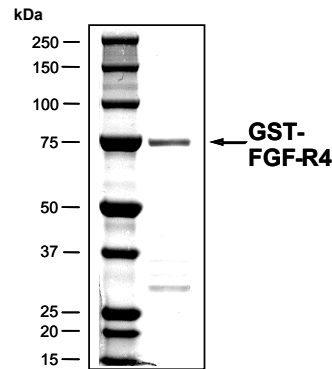
• Assay conditions:

60 mM HEPES-NaOH, pH 7.5
3 mM MgCl₂
3 mM MnCl₂
3 µM Na-orthovanadate
1.2 mM DTT
2.5 µg / 50 µl PEG_{20,000}
ATP (variable)
Substrate: Poly(Glu,Tyr)_{4,1} 2 µg / 50 µl
Recombinant FGF-R4: 100 ng / 50 µl

• Filter binding assay
MAFC membrane (Millipore)

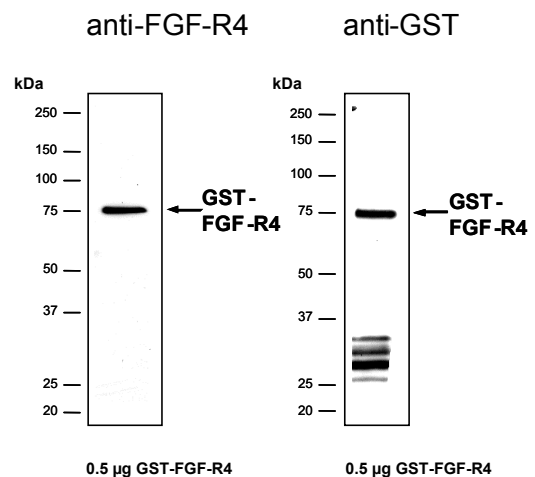
Specific activity: 47 pmol/µg×min

Coomassie stain:



2.0 µg GST-FGF-R4

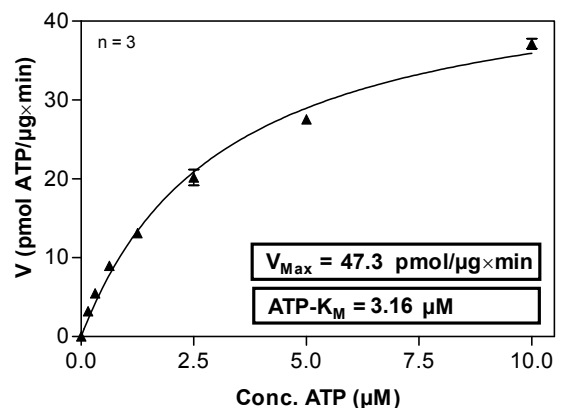
Western blot analysis:



0.5 µg GST-FGF-R4

0.5 µg GST-FGF-R4

Determination of K_m value for ATP:



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FGF-R4 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYE	HLERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHHG	RRRASVAAGI	240
241	LVPRGSPGLD	GICSIEEFRP	PWELRGQALH	GRHPRPPATV	QKLSRFPLAR	QFSLESGSSG	300
301	KSSSSLVRGV	RLSSSGPALL	AGLVSLDLPL	DPLWEFPRDR	LVLGKPLGEG	CFGQVVRAEA	360
361	FGMDPARPDQ	ASTVAVKMLK	DNASDKDLAD	LVSEMEVMKL	IGRHKNIINL	LGVCTQEGPL	420
421	YVIVECAAKG	NLREFLRARR	PPGPDLSPDG	PRSSEGPLSF	PVLVSCAYQV	ARGMQYLESR	480
481	KCIHRDLAAR	NVLVTEDNVM	KIADFGLARG	VHHIDYKKT	SNGRLPVKWM	APEALFDRVY	540
541	THQSDVWSFG	ILLWEIFTLG	GSPYPGIPVE	ELFSLLRREGH	BLMDRPPHCPP	ELYGLMRECV	600
601	HAAPSQRPTF	KQLVEALDKV	LLAVSEEYLD	LRLTFGPYSP	SGGDASSTCS	SSDSVFSHDP	660
661	LPLGSSSFPF	QSGVQT					720

1-218: GST Red: HIS6-tag Pink: Thrombin blue: FGF-R4 fragment

FGF-R4 wt ¹ amino acid sequence							
1	MRLLLALLGV	LLSVPGPPVL	SLEASEEVEL	EPCLAPSLEQ	QEQELTVALG	QPVRLCCGRA	60
61	ERGGHWYKEG	SRLAPAGRVR	GWRGRLEIAS	FLPEDAGRYL	CLARGSMIVL	QNLTLITGDS	120
121	LTSSNDDEDP	KSHRDPSNRH	SYQQQAPYWT	HPQRMEKKLH	AVPAGNTVKF	RCPAAGNPTP	180
181	TIRWLKDGQA	FHGENRIGGI	RLRHQHWSLV	MESVVPDRG	TYTCLVENAV	GSIRYNYLLD	240
241	VLERSPHRPI	LQAGLPANTT	AVVGSDEVLL	CKVYSDAQP	IQWLKHIVIN	GSSFGADGFP	300
301	YVQVLKTADI	NSSEVEVLYL	RNVSAEDAGE	YTCLAGNSIG	LSYQSAWLT	LPEEDPTWTA	360
361	AAPEARYTDI	ILYASGSLAL	AVLLLLLAGLY	RGQALHGRHP	RPPATVQKLS	RFPLARQFSL	420
421	ESGSSGKSSS	SLVRGVLSS	SGPALLAGLV	SLDLPLDPLW	EFPRDLVLG	KPLGEGCFGQ	480
481	VVRAEAFGMD	PARPDQASTV	AVKMLKDNAS	DKDLADLVSE	MEVMKLIGRH	KNIINLLGVC	540
541	TQEGPLYVIV	ECAAAGNLRE	FLRARRPPGP	DLSPDGPRSS	EGPLSFPVLV	SCAYQVARGM	600
600	QYLESRKCIIH	RDLAARNVLV	TEDNVMKIAD	FGLARGVHHI	DYYKKTNGR	LPVKWMAPEA	660
661	LFDRVYTHQS	DVWSFGILLW	EIFTLGGSPY	PGIPVEELFS	LLREGHRMDR	PPHCPPELYG	720
721	LMRECWHAAP	SQRPTFKQLV	EALDKVLLAV	SEEYLDLRLT	FGPYSPSGGD	ASSTCSSSDS	780
781	VFSDHPLPLG	SSSFPGSGV	QT				840

blue: FGF-R4 sequence expressed in fusionprotein

¹NCBI/Protein accession number NP_002002.3

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