Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ VEGFR2

vascular endothelial growth factor receptor 2

Recombinant Human Active Protein Kinase

HGNC Symbol: KDR

Synonyms: FLK1, VEGFR, CD309

Product No.: 0096-0000-1

Lot: 016

Description: Human VEGFR2, C-terminal fragment, amino acids D_{807}-V_{1356} (as in NCBI/Protein entry NP_002244.1), N-terminal GST fusion protein, expressed in Sf9 insect cells

Product identity: VEGFR2 Lot 016, was confirmed as VEGFR2 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 87,095 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

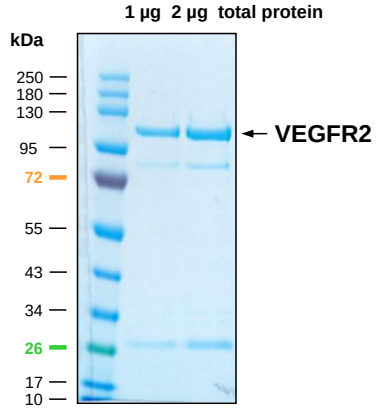
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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!

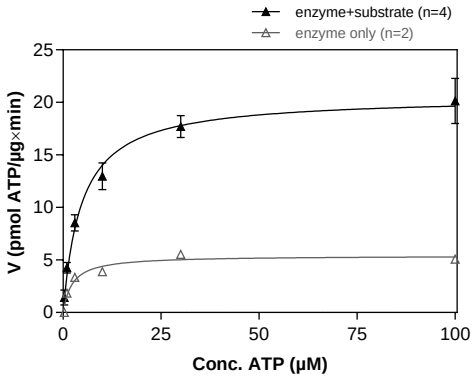
Protein concentration: 0.317 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 21 pmol/µg x min
ATP-K_M: 4.7 µM

VEGFR2 Lot 016: Coomassie stain



VEGFR2 Lot 016: 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: $Poly(Glu:Tyr)_{4:1}$ 20 µg/ml
  Kinase: 4 µg/ml
- Filter binding assay

MSFC membrane (Millipore)

Additional assay technology:
VEGFR2 Lot 016 was also successfully tested by Reaction Biology 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

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VEGFR2

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GST-VEGF-R2 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 **PDELPLDEHC ERLPYDASKW**	240
241	**EFPRDRLKLG KPLGRGAFGQ VIEADAFGID KTATCRTVAV KMLKEGATHS EHRALMSELK**	300
301	**ILIHIGHHLN VVNLLGACTK PGGPLMVIVE FCKFGNLSTY LRSKRNEFVP YKTKGARFRQ**	360
361	**GKDYVGAIPV DLKRRLDSIT SSQSSASSGF VEEKSLSDVE EEEAPEDLYK DFLTLEHLIC**	420
421	**YSFQVAKGME FLASRKCIHR DLAARNILLS EKNVVKICDF GLARDIYKDP DYVRKGDARL**	480
481	**PLKWMAPETI FDRVYTIQSD VWSFGVLLWE IFSLGASPYP GVKIDEEFCR RLKEGTRMRA**	540
541	**PDYTTPEMYQ TMLDCWHGEP SQRPTFSELV EHLGNLLQAN AQQDGKDYIV LPISETLSME**	600
601	**EDSGLSLPTS PVSCMEEEEV CDPKFHYDNT AGISQYLQNS KRKSRPVSVK TFEDIPLEEP**	660
661	**EVKVIPDDNQ TDSGMVLASE ELKTLEDRTK LSPSFGGMVP SKSRESVASE GSNQTSGYQS**	720
721	**GYHSDDTDTT VYSSEEAELL KLIEIGVQTG STAQILQPDS GTTLSSPPV**	780

1-218: GST **blue**: VEGF-R2 fragment

VEGF-R2 wt[1] Amino Acid Sequence

1	MQSKVLLAVA LWLCVETRAA SVGLPSVSLD LPRLSIQKDI LTIKANTTLQ ITCRGQRDLD	60
61	WLWPNNQSGS EQRVEVTECS DGLFCKTLTI PKVIGNDTGA YKCFYRETDL ASVIYVYVQD	120
121	YRSPFIASVS DQHGVVYITE NKNKTVVIPC LGSISNLNVS LCARYPEKRF VPDGNRISWD	180
181	SKKGFTIPSY MISYAGMVFC EAKINDESYQ SIMYIVVVVG YRIYDVVLSP SHGIELSVGE	240
241	KLVLNCTART ELNVGIDFNW EYPSSKHQHK KLVNRDLKTQ SGSEMKKFLS TLTIDGVTRS	300
301	DQGLYTCAAS SGLMTKKNST FVRVHEKPFV AFGSGMESLV EATVGERVRI PAKYLGYPPP	360
361	EIKWYKNGIP LESNHTIKAG HVLTIMEVSE RDTGNYTVIL TNPISKEKQS HVVSLVVYVP	420
421	PQIGEKSLIS PVDSYQYGTT QTLTCTVYAI PPPHHIHWYW QLEEECANEP SQAVSVTNPY	480
481	PCEEWRSVED FQGGNKIEVN KNQFALIEGK NKTVSTLVIQ AANVSALYKC EAVNKVGRGE	540
541	RVISFHVTRG PEITLQPDMQ PTEQESVSLW CTADRSTFEN LTWYKLGPQP LPIHVGELPT	600
601	PVCKNLDTLW KLNATMFSNS TNDILIMELK NASLQDQGDY VCLAQDRKTK KRHCVVRQLT	660
661	VLERVAPTIT GNLENQTTSI GESIEVSCTA SGNPPPQIMW FKDNETLVED SGIVLKDGNR	720
721	NLTIRRVRKE DEGLYTCQAC SVLGCAKVEA FFIIEGAQEK TNLEIIILVG TAVIAMFFWL	780
781	LLVIILRTVK RANGGELKTG YLSIVM**DPDE LPLDEHCERL PYDASKWEFP RDRLKLGKPL**	840
841	**GRGAFGQVIE ADAFGIDKTA TCRTVAVKML KEGATHSEHR ALMSELKILI HIGHHLNVVN**	900
901	**LLGACTKPGG PLMVIVEFCK FGNLSTYLRS KRNEFVPYKT KGARFRQGKD YVGAIPVDLK**	960
961	**RRLDSITSSQ SSASSGFVEE KSLSDVEEEE APEDLYKDFL TLEHLICYSF QVAKGMEFLA**	1020
1021	**SRKCIHRDLA ARNILLSEKN VVKICDFGLA RDIYKDPDYV RKGDARLPLK WMAPETIFDR**	1080
1081	**VYTIQSDVWS FGVLLWEIFS LGASPYPGVK IDEEFCRRLK EGTRMRAPDY TTPEMYQTML**	1140
1141	**DCWHGEPSQR PTFSELVEHL GNLLQANAQQ DGKDYIVLPI SETLSMEEDS GLSLPTSPVS**	1200
1201	**CMEEEEVCDP KFHYDNTAGI SQYLQNSKRK SRPVSVKTFE DIPLEEPEVK VIPDDNQTDS**	1260
1261	**GMVLASEELK TLEDRTKLSP SFGGMVPSKS RESVASEGSN QTSGYQSGYH SDDTDTTVYS**	1320
1321	**SEEAELLKLI EIGVQTGSTA QILQPDSGTT LSSPPV**	1380

blue: VEGF-R2 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_002244.1

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