

RET E762Q

Ret proto-oncogene

Recombinant Human Active Protein Kinase

HGNC Symbol: RET

Synonyms: CDHF12, CDHR16, HSCR1, MEN2A, MEN2B, MTC1, PTC, RET51, RET-ELE1

Product No.: 1093-0000-1

Lot: 001

Description: Human RET E762Q, C-terminal fragment, amino acids H₆₅₈-S₁₁₁₄ (as in NCBI/Protein entry NP_066124.1) with a E762Q mutation, N-terminal GST-HIS₆ fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: RET E762Q Lot 001, was confirmed as RET E762Q by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 80,435 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

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

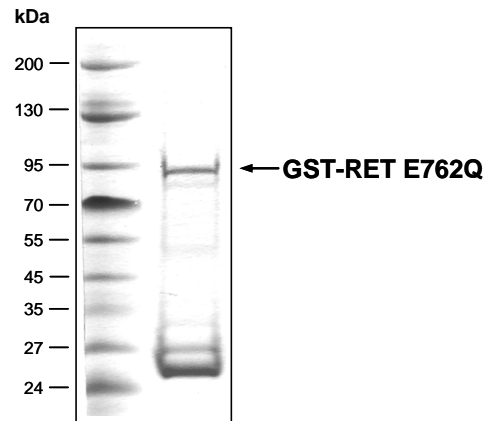
Storage temperature: -80°C
Avoid repeated freeze-thaw cycles!

Protein concentration: 0.134 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

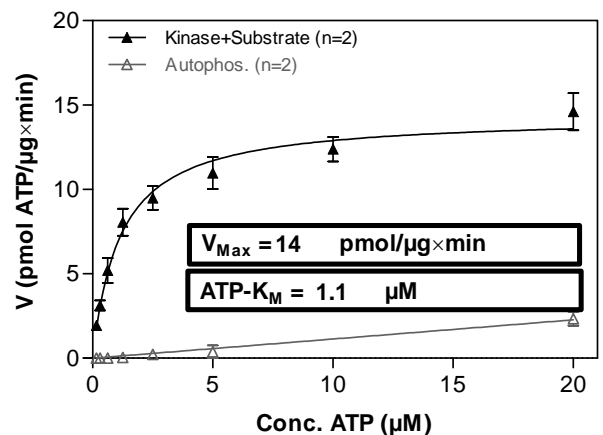
Specific activity: 14 pmol/µg×min
ATP-K_M: 1.1 µM

**RET E762Q Lot 001:
Coomassie stain**



2.0 µg GST-RET E762Q

**RET E762Q 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 MgCl₂
 - 3 mM MnCl₂
 - 3 µM Na-orthovanadate
 - 1.2 mM DTT
 - 50 µg / ml PEG_{20,000}
 - ATP (variable)
 - Substrate: TRK-C-derived Peptide (R11-VYSTDYRRLFNPS), 80 µg / ml
 - RET E762Q: 1.0 µg / ml
- Filter binding assay
 - MSPH membrane (Millipore)

Additional assay technology: RET E762Q Lot 001

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

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RET E762Q Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSM	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLP EML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHGG	RDSLEVLVFG	240
241	PLAMGARGRH	CYHKFAHKPP	ISSAEMTFRR	PAQAFVVSYS	SSGARRPSLD	SMENQVSVDA	300
301	FKILEDPKWE	FPRKNLVLGK	TLGEGEFGKV	VKATAFHLKG	RAGYTTVAVK	MLK ^Q NASPSE	360
361	LRDLLSEFNV	LKQVNHPhVI	KLYGACSQDG	PLLLIVEYAK	YGSLRGFLRE	SRKVGPYLG	420
421	SGGSRNSSSL	DHPDERALTM	GDLISFAWQI	SQGMQYLAEM	KLVRDLAAR	NILVAEGRKM	480
481	KISDFGLSRD	VYEEDSYVKR	SQGRI PVKWM	AIESLFDHIY	TTQSDVWSFG	VLLWEIVTLG	540
541	GNPYPGIPPE	RLFNLLKTGH	RMERPDNCSE	EMYRLMLQCW	KQEPDKRPVF	ADISKDLEKM	600
600	MVKRRDYL DL	AASTPSDSL I	YDDGLSEEET	PLVDCNNAPL	PRALPSTWIE	NKLYGMSDPN	660
661	WPGESPVPLT	RADGTNTGFP	RYPNDLSVYAN	WMLSPSAAKL	MDTFDS		720

1-218: GST Red: HIS6-tag Green: 3C blue:RET fragment ^Q: E762Q point mutation

RET wt ¹ amino acid sequence							
1	MAKATSGAAG	LRLLLLLLLP	LLGKVALGLY	FSRDAYWEKL	YVDQAAGTPL	LYVHALRDAP	60
61	EEVPSFRLGQ	HLYGTYRTRL	HENNWICIQE	DTGLLYLNRS	LDHSSWEKLS	VRNRGFPLLT	120
121	VYLVKFLSPT	SLREGECQWP	GCARVYFSFF	NTSFPACSSL	KPRELCFPET	RPSFRIRENR	180
181	PPGTFHQFRL	LPVQFLCPNI	SVAYRLLEGE	GLPFRCAPDS	LEVSTRWALD	REQREKYELV	240
241	AVCTVHAGAR	EEVVMVFPV	TVYDEDD SAP	TFPAGVDTAS	AVVEFKRKED	TVVATLRVFD	300
301	ADVVPASGEL	VRRYTSTLLP	GDTWAQQTFR	VEHWPNETSV	QANGSFVRAT	VHDYRLVLNR	360
361	NLSISENRTM	QLAVLVNDS	FQPGAGVLL	LHFNVS VLPV	SLHLPSTYSL	SVSRRARRFA	420
421	QIGKVCVENC	QAFSGINVQY	KLHSSGANCS	TLGVVTS AED	TSGILFVNDT	KALRRPKCAE	480
481	LHYMVVATDQ	QTSRQAQAQL	LVTVEGSYVA	EEAGCPLS CA	VSKRRL ECEE	CGGLGSPTGR	540
541	CEWRQGDGKG	ITRNFSTCSP	STKTCPDGHC	DVVETQDINI	CPQDCLR GSI	VGGHEPGEPR	600
600	GIKAGYGTCN	CFPEEEKFC	EPEDIQDPLC	DEL CRTVIAA	AVLFSFIVSV	LLSAFCIHCY	660
661	HKFAHKPPIS	SAEMTFRRPA	QAFVVSYS	GARRPSLDSM	ENQVSVDAFK	ILEDPKWEFP	720
721	RKNLVLGKTL	GEGEFGKVK	ATAFHLKGRA	GYTTVAVKML	KENASPSEL R	DLLSEFNVLK	780
781	QVNHPhVIKL	YGACSQDGPL	LLIVEYAKYG	SLRGFLRESR	KVGPYLGSG	GSRNSSSLDH	840
841	PDERALTMGD	LISFAWQISQ	GMQYLAEMKL	VHRDLAARNI	LVAEGRKMKI	SDFGLSRDVY	900
901	EEDSYVKRSQ	GRIPVKWMAI	ESLFDHIYTT	QSDVWSFGVL	LWEIVTLGGN	PYPGIPPERL	960
961	FNLLKTGHRM	ERPDNCSEEM	YRLMLQCWKQ	EPDKRPVFAD	ISKDLEKMMV	KRRDYDLAA	1020
1021	STPSDSL IYD	DGLSEEETPL	VDCNNAPLPR	ALPSTWIENK	LYGMSDPNWP	GESPVPLTRA	1080
1081	DGTNTGFP RY	PND SVYANWM	LSPSAAKLMD	TFDS			1140

blue: RET sequence expressed in fusionprotein Red: variant in fusionprotein

¹NCBI/Protein accession number NP_066124.1