

RET R813Q

ret proto-oncogene

Recombinant Human Active Protein Kinase

HGNC Symbol: RET

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

Product No.: 1097-0000-1

Lot: 001

Description: Human RET R813Q, C-terminal fragment, amino acids H₆₅₈-S₁₁₁₄ (as in [NCBI/Protein](#) entry NP_066124.1) with a R₈₁₃Q mutation, N-terminal GST-HIS₆ fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: RET R813Q Lot 001, was confirmed as RET R813Q by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 80,408 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: 0.203 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

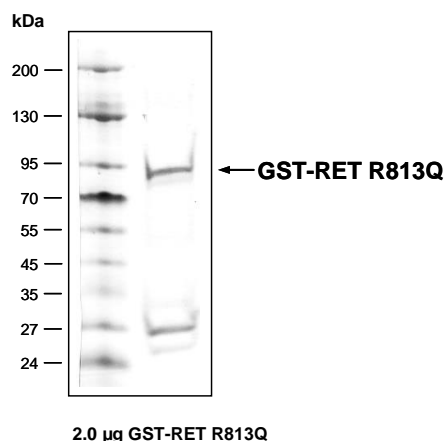
Biochemical Parameters:

Specific kinase activity (P_i transfer): 15 pmol/µg × min
ATP-K_M: 1.7 µM

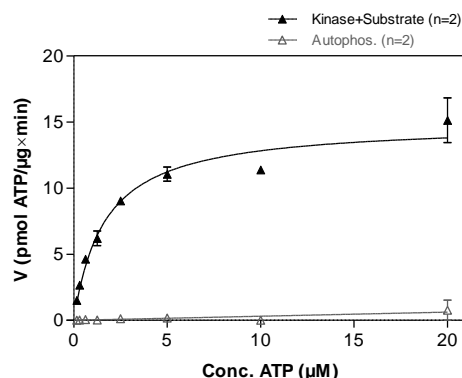
Additional assay technology:

RET R813Q Lot 001 was also successfully tested by ProQinase 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

RET R813Q Lot 001: Coomassie stain



RET R813Q 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 80 µg/ml
 - Kinase: 1 µg/ml
- Filter binding assay
MSPH membrane (Millipore)

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GST-RET R813Q Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG HHHHHG	RDS LEVLFCG	240
241	PLAMGARGRH	CYHKFAHKPP	ISSAEMTFRR	PAQAFPVSYS	SSGARRPSLD	SMENQVSVDA	300
301	FKILEDPKWE	FPRKNLVLGK	TLGEGEFGKV	VKATAFHLKG	RAGYTTVAVK	MLKENASPSE	360
361	LRDLLSEFNV	LKQVNHPHVI	KLYGACSQDG	PLLLIVEYAK	YGSLQGFLE	SRKVGPGYLG	420
421	SGGSRNSSSL	DHPDERALTM	GDLISFAWQI	SQGMQYLAEM	KLVHRDLAAR	NILVAEGRKM	480
481	KISDFGLSRD	VYEEDSYVKR	SQGRIPVKWM	AIESLFDHIY	TTQSDVWSFG	VLLWEIVTLG	540
541	GNPYPGIPPE	RLFNLLKTGH	RMERPDNCSE	EMYRLMLQCW	KQEPDKRPVF	ADISKDLEKM	600
600	MVKRRDYLDL	AASTPSDSL	YDDGLSEET	PLVDCNNAPL	PRALPSTWIE	NKLYGMSDPN	660
661	WPGESPVPPLT	RADGTNTGFP	RYPNDSVYAN	WMLSPSAAKL	MDTFDS		720

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: RET fragment **boxed**: R813Q mutation

RET wt ¹ Amino Acid Sequence							
1	MAKATSGAAG	LRLLLLLLLP	LLGKVALGLY	FSRDAYWEKL	YVDQAAGTPL	LYVHALRDAP	60
61	EEVPSFRLGQ	HLYGTYRTRL	HENNWICIQE	DTGLLYLNRS	LDHSSWEKLS	VRNRGFPLLT	120
121	VYLKVFLSPT	SLREGECQWP	GCARVYFSFF	NTSFPACSSL	KPRELCFPET	RPSFRIENR	180
181	PPGTFHQFRL	LPVQFLCPNI	SVAYRLLEGE	GLPFRCAPDS	LEVSTRWALD	REQREKYELV	240
241	AVCTVHAGAR	EEVVMVFPFV	TVYDEDDSAF	TFPAGVDTAS	AVVEFKRKED	TVVATLRVFD	300
301	ADVVPASGEL	VRRYTSTLLP	GDTWAQQTFR	VEHWPNETSV	QANGSFVRAT	VHDYRLVLNR	360
361	NLSISENRTM	QLAVLVNDS	FQGGPAGVLL	LHFNVSVPV	SLHLPSTYSL	SVSRRARRFA	420
421	QIGKVCVENC	QAFSGINVQY	KLHSSGANCS	TLGVVTS AED	TSGILFVNDT	KALRRPKCAE	480
481	LHYMVVATDQ	QTSRQAQAQL	LVTVEGSYVA	EEAGCPLSCA	VSKRRLECEE	CGGLGSP TGR	540
541	CEWRQGDGKG	ITRNFSTCSP	STKTCPDGHC	DVVETQDINI	CPQDCLRGS	VGGHEPGEPR	600
600	GIKAGYGTCN	CFPEEEKFC	EPEDIQDPLC	DELCRTVIAA	AVLFSFIVSV	LLSAFCI HCY	660
661	HKFAHKPPIS	SAEMTFRRPA	QAFPVSYS	GARRPSLDSM	ENQVSVDAFK	ILEDPKWEFF	720
721	RKNLVLGKTL	GEGEFGKVVK	ATAFHLKGRA	GYTTVAVKML	KENASPSELR	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	ERPDCNSEEM	YRLMLQCWKQ	EPDKRPVFAD	ISKDLEKMMV	KRRDYDLAA	1020
1021	STPSDSLIDY	DGLSEETPL	VDCNNAPLPR	ALPSTWIENK	LYGMSDPNWP	GESPVPLTRA	1080
1081	DGTNTGFPRY	PNSVYANWM	LSPSAAKLMD	TFDS			1140

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

¹[NCBI/Protein](https://www.ncbi.nlm.nih.gov/protein/NP_066124.1) accession number NP_066124.1

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