

ProQinase™ RET V804M

ret proto-oncogene

Recombinant Human Active Protein Kinase

HGNC Symbol: RET

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

Product No.: 1095-0000-1

Lot: 003

Description: Human RET, C-terminal fragment, amino acids H₆₅₈-S₁₁₁₄ (as in [NCBI/Protein](#) entry NP_066124.1) with point mutation V804M, N-terminal GST-HIS₆ fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: RET V804M Lot 003, has been verified by mass spectrometry LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 80468 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

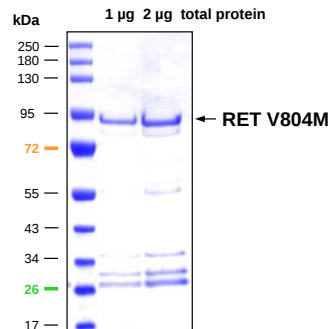
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10 µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.170 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

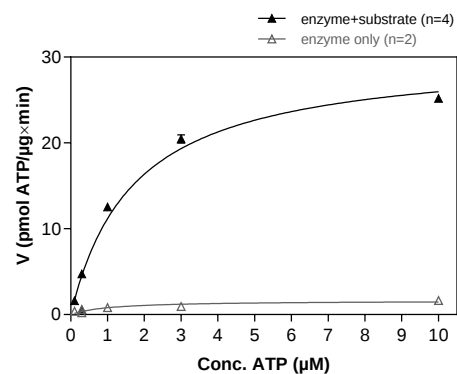
Biochemical Parameters:

Specific kinase activity (P_i transfer): 30 pmol/µg*min
ATP-K_M: 1.7 µM

**RET V804M Lot 003:
Coomassie stain**



**RET V804M Lot 003:
Determination of V_{max} and K_M value for ATP**



- 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: TRKC derived peptide 20 µg/ml
 - Kinase: 2 µg/ml

Assay technology:
Radiometric filter binding assay
MSIP membrane (96 well plate, Millipore)

Recombinant Proteins

Sequence information

GST-RET V804M Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLIERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPIQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHGG	RDSLEVLFGG	240
241	PLAMGARGRH	CYHKFAHKPP	ISSAEMTFRR	PAQAFVVSYS	SSGARRPSLD	SMENQVSVDA	300
301	FKILEDPKWE	FPRKNLVLGK	TLGEGEFGKV	VKATAFHLKG	RAGYTTVAVK	MLKENASPSE	360
361	LRDLLSEFNV	LKQVNHPhVI	KLYGACSQDG	PLLLIMEYAK	YGSIRGFLRE	SRKVGPGYLG	420
421	SGGSRNSSSL	DHPDERALTM	GDLISFAWQI	SQGMQYLAEM	KLVRDLAAR	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	WPGESPVLPT	RADGTNTGFP	RYPNDSVYAN	WMLSPSAAKL	MDTFDS		720

11-218: GST Red: HIS6-tag Green: 3C cleavage site blue: RET fragment boxed: V804M mutation

RET wt ¹ Amino Acid Sequence							
1	MAKATSGAAG	LRLLLLLLLP	LLGKVALGLY	FSRDAYWEKL	YVDQAAGTPL	LYVHALRDAP	60
61	EEVPSFRLGQ	HLGYTYRTRL	HENNWICIQE	DTGLLYLNRS	LDHSSWEKLS	VRNRGFPLLT	120
121	VYLKVFLSPT	SLREGECQWP	GCARVYFSFF	NTSFPACSSL	KPRELCFPET	RPSFRIENR	180
181	PPGTFHQFRL	LPVQFLCPNI	SVAYRLLEGE	GLPFRCAPDS	LEVSTRWALD	REQREKYELV	240
241	AVCTVHAGAR	EEVVMVPFPV	TVYDEDDSDP	TFPAGVDTAS	AVVEFKRKED	TVVATLRVFD	300
301	ADVVPASGEL	VRRYTSTLLP	GDTWAQQTFR	VEHWPNETSV	QANGSFVRAT	VHDYRLVLNR	360
361	NLSISENRTM	QLAVLVNDS	FQGGPGAGVLL	LHFNVSVLPV	SLHLPSYSL	SVSRRARRFA	420
421	QIGKVCVENC	QAFSGINVQY	KLHSSGANCS	TLGVVTS AED	TSGILFVNDT	KALRRPKCAE	480
481	LHYMVVATDQ	QTSRQAQAQL	LVTVEGSYVA	EEAGCPLSCA	VSKRRLECEE	CGGLGSPTGR	540
541	CEWRQGDGKG	ITRNFSTCSP	STKTCPDGHC	DVETQDINI	CPQDCLRGSI	VGGHEPGEPR	600
600	GIKAGYGTGN	CFPEEEKCFC	EPEDIQDPLC	DEL CRTVIAA	AVLFSFIVSV	LLSAFCIHCY	660
661	HKFAHKPPIS	SAEMTFRRPA	QAFVVSYS	GARRPSLDSM	ENQVSVDAFK	ILEDPKWEFP	720
721	RKNLVLGKTL	GEGEFGKVVK	ATAFHLKGRA	GYTTVAVKML	KENASPSEL	DLLEFNVK	780
781	QVNHPhVIKL	YGACSQDGPL	LLIIVEYAKYG	SLRGFLRESR	KVGPYGLSG	GSRNSSSLDH	840
841	PDERALTMGD	LISFAWQISQ	GMQYLAEMKL	VHRDLAARNI	LVAEGRMKI	SDFGLSRDVF	900
901	EEDSYVKRSQ	GRIPVKWMAI	ESLFDHIYTT	QSDVWSFGVL	LWEIVTLGNN	PYPGIPPERL	960
961	FNLLKTGHRM	ERPDNCSEEM	YRLMLQCWKQ	EPDKRPVFAD	ISKDLEKMMV	KRRDYLDLAA	1020
1021	STPSDSLIDY	DGLSEETPL	VDCNNAPLPR	ALPSTWIENK	LYGMSDPNWP	GESPVLTRA	1080
1081	DGTNTGFPRY	PNDVSVYANWM	LSPSAAKLMD	TFDS			1140

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

¹NCBI/Protein accession number NP_066124.1

Recombinant Proteins