

ProQinase™ RET V804E

ret proto-oncogene

Recombinant Human Active Protein Kinase

HGNC Symbol: RET

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

Product No.: 1377-0000-1

Lot: 002

Description: Human RET V804E, C-terminal fragment, amino acids H₆₅₈-S₁₁₁₄ (as in [NCBI/Protein](#) entry NP_066124.1) with a V804E mutation, N-terminal GST-HIS₆ fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: RET V804E Lot 002, was confirmed as RET by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 80,466 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: his 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: 0.167 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

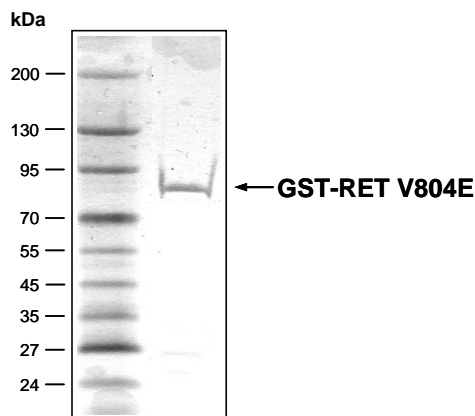
Biochemical Parameters:

Specific kinase activity (P_i transfer): 228 pmol/µg × min
ATP-K_M: 2.9 µM

Additional assay technology:

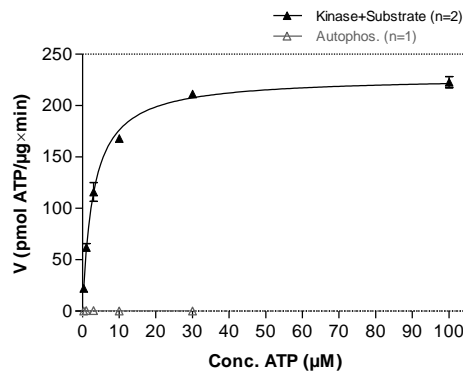
RET V804E Lot 002 was also successfully tested by Reaction Biology for the use with the ADP-Glo™ Kinase assay from Promega ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

RET V804E Lot 002: Coomassie stain



2.0 µg GST-RET V804E

RET V804E Lot 002: 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: TRK-C-derived Peptide 80 µg/ml
Kinase: 0.5 µg/ml
- Filter binding assay
MSPH membrane (Millipore)

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GST-RET V804E Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDKVLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHG	RDSLEVLFCG	240
241	PLAMGARGRH	CYHKFAHKPP	ISSAEMTFRR	PAQAFPVSYS	SSGARRPSLD	SMENQVSVDA	300
301	FKILEDPKWE	FPRKNLVLGK	TLGEGEFGKV	VKATAFHLKG	RAGYTTVAVK	MLKENASPSE	360
361	LRDLLSEFNV	LKQVNHPHVI	KLYGACSQDG	PLLLIEYAK	YGSLRGFLRE	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	WPGESPVPLT	RADGTNTGFP	RYPNDSVYAN	WMLSPSAAKL	MDTFDS		720

1-218: GST Red: HIS6-tag Green: 3C cleavage site blue: RET fragment boxed: V804E

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	SVAYRLLIGE	GLPFRCAPDS	LEVSTRWALD	REQREKYELV	240
241	AVCTVHAGAR	EEVVMVPPFV	TVYDEDDSA	TFPAGVDTAS	AVVEFKRKED	TVVATLRVFD	300
301	ADVVPASGEL	VRRYTSTLLP	GDTWAQQTFR	VEHWPNETSV	QANGSFVRAT	VHDYRLVLNR	360
361	NLSISENRTM	QLAVLVNDS	FQGPAGVLL	LHFNVSVLPV	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	IVGGHEPGEPR	600
600	GIKAGYGTGN	CFPEEEKCFC	EPEDIQDPLC	DEL CRTVIAA	AVLFSFIVSV	LLSAFCIHCY	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	SDFGLSRD VY	900
901	EEDSYVKRSQ	GRIPVKWMAI	ESLFDHIYTT	QSDVWSFGVL	LWEIVTLGGN	PYPGIPPERL	960
961	FNLLKTGHRM	ERPDCSEEM	YRLMLQCWKQ	EPDKRPVFAD	ISKDLEKMMV	KRRDYLDLAA	1020
1021	STPSDSL IYD	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](#) accession number NP_066124.1