

ProQinase™ RET G691S

ret proto-oncogene

Recombinant Human Active Protein Kinase

HGNC Symbol: RET

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

Product No.: 1091-0000-1

Lot: 002

Description: Human RET G691S, C-terminal fragment, amino acids H658-S1114 (as in [NCBI/Protein](#) entry NP_066124.1) with a G691S mutation, N-terminal GST-HIS6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: RET G691S 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: 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

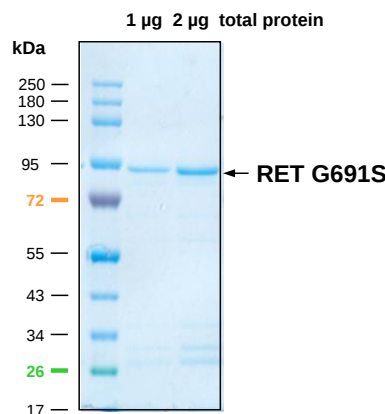
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10 µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.299 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

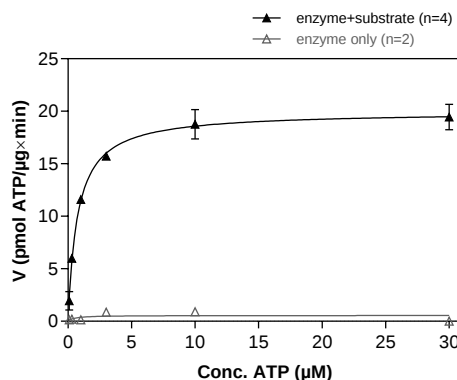
Biochemical Parameters:

Specific kinase activity (P_i transfer): 20 pmol/µg x min
ATP-K_M: 0.7 µM

RET G691S Lot 002: Coomassie stain



RET G691S 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 10 µg/ml
Kinase: 1 µg/ml

- Filter binding assay
MSPI membrane (Millipore)

Additional assay technology:

RET G691S 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 G691S

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GST-RET G691S Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPIQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHG	RDSLEVLFGQ	240
241	PLAMGARGRH	CYHKFAHKPP	ISSAEMTFRR	PAQAFPVSYS	SSSARRPSLD	SMENQVSVDA	300
301	FKILEDPKWE	FPRKNLVLGK	TLGEGEFGKV	VKATAFHLKG	RAGYTTVAVK	MLKENASPSE	360
361	LRDLLSEFNV	LKQVNHPIVI	KLYGACSQDG	PLLLIVEYAK	YGSIRGFLRE	SRKVGPGYLG	420
421	SGGSRNSSSL	DHPDERALTM	GDLISFAWQI	SQGMQYLAEM	KLVRDLAAR	NILVAEGRKM	480
481	KISDFGLSRD	VYEEDSYVKR	SQGRIPVKWM	AIESLFDHIY	TTQSDVWSFG	VLLWEIVTLG	540
541	GNPYPGIPPE	RLEFNLLKTGH	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: G691S mutation

RET wt ¹ Amino Acid Sequence							
1	MAKATSGAAG	LRLLLLLLLP	LLGKVALGLY	FSRDAYWEKL	YVDQAAGTPL	LYVHALRDAP	60
61	EEVPSFRLGQ	HLYGTYRTRL	HENNWICIQE	DTGLLYLNRS	LDHSSWEKLS	VRNRGFPLLT	120
121	VYLKVFSLPT	SLREGEQWP	GCAVYFSFF	NTSFPACSSL	KPRELCFPET	RPSFRIRENR	180
181	PPGTFHQFRL	LPVQFLCPNI	SVAYRLLEGE	GLPFRCAPDS	LEVSTRWALD	REQREKYELV	240
241	AVCTVHAGAR	EEVVMVPFPV	TVYDEDDSD	TFPAGVDTAS	AVVEFKRKED	TVVATLRVFD	300
301	ADVVPASGEL	VRRYTSTLLP	GDTWAQQTFR	VEHWPNETSV	QANGSFVRAT	VHDYRLVLNR	360
361	NLSISENRTM	QLAVLVNDS	FQGPAGAVLL	LHFNVSVPV	SLHLPLSTYSL	SVSRRARRFA	420
421	QIGKVCVENC	QAFSGINVQY	KLHSSGANCS	TLGVVTS AED	TSGILFVNDT	KALRRPKCAE	480
481	LHYMVVATDQ	QTSRQAQQL	LVTVEGSYVA	EEAGCPLSCA	VSKRRLECEE	CGGLGSPTGR	540
541	CEWRQGDGKG	ITRNFSTCSP	STKTCPDGHC	DVVETQDINI	CPQDCLRGS	VGGHEPGEPR	600
600	GKAGYGTCTN	CFPEEEKCFC	EPEDIQDPLC	DEL CRTVIAA	AVLFSFIVSV	LLSAFCIHCY	660
661	HKFAHKPPIS	SAEMTFRRPA	QAFPVSYS	SSSARRPSLD	SMENQVSDAFK	ILEDPKWEFF	720
721	RKNLVLGKTL	GEGEFGKVVK	ATAFHLKGRA	GYTTVAVKML	KENASPSEL	DLLEFNV LK	780
781	QVNHPIVIKL	YGACSQDGPL	LLIVEYAKYG	SLRGFLRESR	KVGPGYLGSG	GSRNSSSLDH	840
841	PDERALTMGD	LISFAWQISQ	GMQYLAEMKL	VHRDLAARNI	LVAEGRKMKI	SDFGLSRDVY	900
901	EEDSYVKRSQ	GRIPVKWMAI	ESLFDHIYTT	QSDVWSFGVL	LWEIVTLGGN	PYPGIPPERL	960
961	FNLLKTGHRM	ERPDNCSEEM	YRLMLQCWKQ	EPDKRPVFAD	ISKDLEKMMV	KRRDYLDLAA	1020
1021	STPSDSL IYD	DGLSEETPL	VDCNNAPLPR	ALPSTWIENK	LYGMSDPNWP	GESPVPLTRA	1080
1081	DGTNTGFPRY	PND SVYANWM	LSPSAAKLMD	TFDS			1140

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

¹NCBI/Protein accession number NP_066124.1