

ProQinase™ RET L730M

ret proto-oncogene

Recombinant Human Active Protein Kinase

HGNC Symbol: RET

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

Product No.: 1736-0000-1

Lot: 001

Description: Human RET, C-terminal fragment, amino acids H₆₅₈-S₁₁₁₄ (as in [NCBI/Protein](#) entry NP_066124.1) with a L730M point mutation, N-terminal GST-HIS₆ fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: RET L730M Lot 001, was confirmed as RET wt by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 80,453 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: 0.436 µg/µl

(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

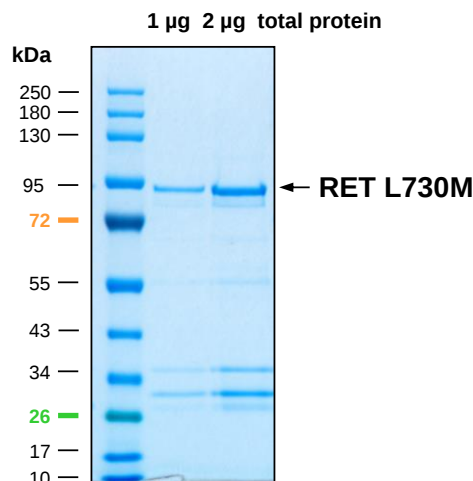
Specific kinase activity (P_i transfer): 14 pmol/µg × min

ATP-K_M: 2.2 µM

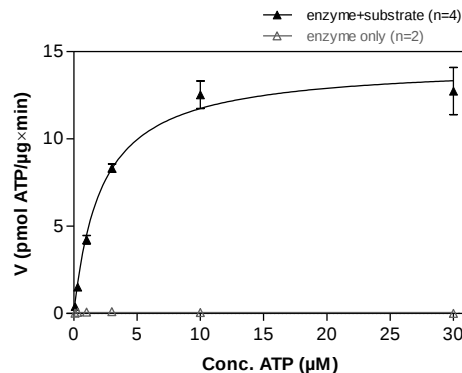
Additional assay technology:

RET L730M Lot 001 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 L730M Lot 001: Coomassie stain



RET L730M Lot 001: 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 5 µg/ml
Kinase: 2 µg/ml
- Filter binding assay
MSIP membrane (Millipore)

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GST-RET L730M Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLIERDEGDK	WRNKKFELGL	EFNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFKDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHG	RDSLEVLFGG	240
241	PLAMGARGRH	CYHKFAHKPP	ISSAEMTFRR	PAQAFVVSYS	SSGARRPSLD	SMENQVSVD	300
301	FKILEDPKWE	FPRKNLVLGK	TMGEFEGKV	VKATAFHLKG	RAGYTTVAVK	MLKENASPSE	360
361	LRDLLSEFNV	LKQVNHPHVI	KLYGACSQDG	PLLLIVEYAK	YGSRLRGFLRE	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
601	MVKRRDYLDL	AASTPSDSL	YDDGLSEET	PLVDCNNAPL	PRALPSTWIE	NKLYGMSDPN	660
661	WPGESPVPPL	RADGTNTGFP	RYPNDSVYAN	WMLSPSAAKL	MDTFDS		720

1-218: GST Red: HIS6-tag Green: 3C cleavage site blue: RET fragment boxed: L730M 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	SLREGECQWP	GCARVYFSFF	NTSFPACSSL	KPRELCFPET	RPSFRIENR	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	FQGPAGVLL	LHFNVSVPV	SLHLPSTYSI	SVSRRARRFA	420
421	QIGKVCVENC	QAFSGINVQY	KLHSSGANCS	TLGVVTSAD	TSGILFVNDT	KALRRPKCAE	480
481	LHYMVVATDQ	QTSRQAQAQL	LVTVEGSYVA	EEAGCPLSCA	VSKRRLECEE	CGGLGSPTGR	540
541	CEWRQGDGKG	ITRNFSTCSP	STKTCPDGHC	DVVETQDINI	CPQDCLRGS	VGGHEPGEPR	600
600	GIKAGYGTCT	CFPEEEKCFC	EPEDIQDPLC	DELCRTVIAA	AVLFSFIVSV	LLSAFCIHCY	660
661	HKFAHKPPIS	SAEMTFRRPA	QAFVSYSSSS	GARRPSLDSM	ENQVSVDFAK	ILEDPKWEFF	720
721	RKNLVLGKTL	GEFEGKVVK	ATAFHLKGRA	GYTTVAVKML	KENASPSEL	DLLSEFNVLK	780
781	QVNHPHVIKL	YGACSQDGPL	LLIVEYAKYG	SLRGFLRESR	KVGPGYLGSG	GSRNSSLSDH	840
841	PDERALTMGD	LISFAWQISQ	GMQYLAEMKL	VHRDLAARNI	LVAEGRMKI	SDFGLSRDVI	900
901	EEDSYVKRSQ	GRIPVKWMAI	ESLFDHIYTT	QSDVWSFGVL	LWEIVTLGN	PYPGIPPERL	960
961	FNLLKTGHRM	ERPDCNSEEM	YRLMLQCWKQ	EPDKRPVFAD	ISKDLEKMMV	KRRDYDLAA	1020
1021	STPSDSLID	DGLSEETPL	VDCNNAPLPR	ALPSTWIENK	LYGMSDPNWP	GESPVPPLTRA	1080
1081	DGTNTGFPRY	PNDVSYANWM	LSPSAAKLMD	TFDS			1140

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

¹NCBI/Protein accession number NP_066124.1