

ProQinase™ RET R749T

Ret proto-oncogene

Recombinant Human Active Protein Kinase

HGNC Symbol: RET

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

Product No.: 1092-0000-1

Lot: 001

Description: Human RET R749T, C-terminal fragment, amino acids H₆₅₈-S₁₁₁₄ (as in NCBI/Protein entry NP_066124.1) with a R749T mutation, N-terminal GST-HIS₆ fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: RET R749T Lot 001, was confirmed as RET R749T by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 80,381 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol

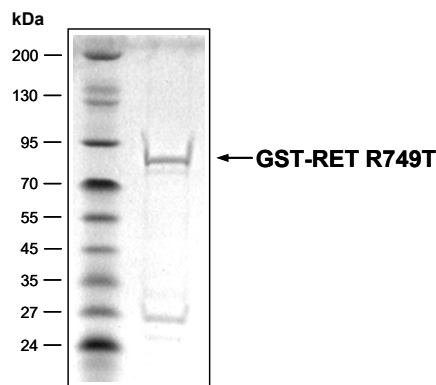
Storage temperature: -80°C
Avoid repeated freeze-thaw cycles!

Protein concentration: 0.219 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

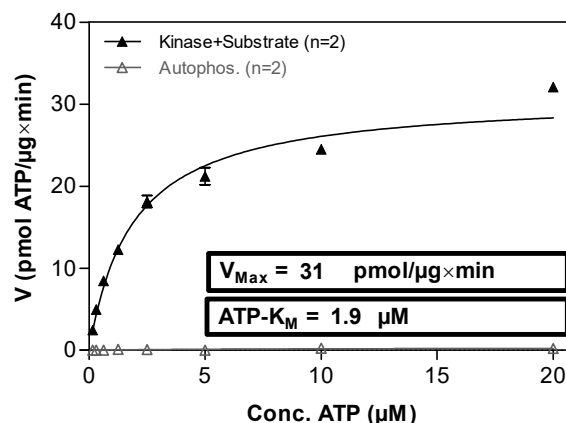
Specific activity: 31 pmol/µg×min
ATP-K_M: 1.9 µM

RET R749T Lot 001: Coomassie stain



2.0 µg GST-RET R749T

RET R749T 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 (R11-VYSTDYRFLNPS), 80 µg / ml
 - RET R749T: 1.0 µg / ml
- Filter binding assay
 - MSPH membrane (Millipore)

Additional assay technology: RET R749T Lot 001

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



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RET R749T 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	KRIEAIQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHG	RDSLEVLFG	240
241	PLAMGARGRH	CYHKFAHKPP	ISSAEMTFRR	PAQAFVVSYS	SSGARRPSLD	SMENQVSVDA	300
301	FKILEDPKWE	FPRKNLVLGK	TLGEGEFQKV	VKATAFHLKG	TAGYTTVAVK	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
600	MVKRRDYLDL	AASTPSDSL	YDDGLSEEET	PLVDCNNAPL	PRALPSTWIE	NKLYGMSDPN	660
661	WPGESPVLPT	RADGTNTGFP	RYPNDSVYAN	WMLSPSAAKL	MDTFDS		720

1-218: GST Red: HIS6-tag Green: 3C blue:RET fragment T: R749T point mutant

RET wt¹ amino acid sequence

1	MAKATSGAAG	LRLLLLLLLP	LLGKVALGLY	FSRDAYWEKL	YVDQAAGTPL	LYVHALRDAP	60
61	EEVPSFRLGQ	HLYGTYRTRL	HENNWICIQE	DTGLLYLNRS	LDHSSWEKLS	VRNRGFPLLT	120
121	VYLKVFLSPT	SLREGEQWP	GCARVYFSFF	NTSFPACSSL	KPRELCFPET	RPSFRIRENR	180
181	PPGTFHQFRL	LPVQFLCPNI	SVAYRLLEGE	GLPFRCAPDS	LEVSTRWALD	REQREKYELV	240
241	AVCTVHAGAR	EEVMVVPFPV	TVYDEDDSD	TFPAGVDTAS	AVVEFKRKED	TVVATLRVFD	300
301	ADVVPASGEL	VRRYTSTLLP	GDTWAQOTFR	VEHWPNETS	QANGSFVRAT	VHDYRLVLNR	360
361	NLSISENRTM	QLAVLVNDS	FQGGAGVLL	LHFNVSVLPV	SLHLPSTYSL	SVSRRARRFA	420
421	QIGKVCVENC	QAFSGINVQY	KLHSSGANCS	TLGVVTS AED	TSGILFVNDT	KALRRPKCAE	480
481	LHYMVVATDQ	QTSRQAQQL	LVTVEGSYVA	EEAGCPLSCA	VSKRRLECEE	CGGLGSP TGR	540
541	CEWRQGDGKG	ITRNFSTCSP	STKTCPDGHC	DVVETQDINI	CPQDCLRGS	VGGHEPGEPR	600
600	GIKAGYGTCTN	CFPEEEKCFC	EPEDIQDPLC	DEL CRTVIAA	AVLFSFIVSV	LLSAFCIHCY	660
661	HKFAHKPPIS	SAEMTFRRPA	QAFVSYSSS	GARRPSLDSM	ENQVSVDAFK	ILEDPKWEFP	720
721	RKNLVLGKTL	GEFEFGKVVK	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	ERPDCNSEEM	YRLMLQCWKQ	EPDKRPVFAD	ISKDLEKMMV	KRRDYLDLAA	1020
1021	STPSDSL IYD	DGLSEEETPL	VDCNNAPLPR	ALPSTWIENK	LYGMSDPNWP	GESPVLPTRA	1080
1081	DGTNTGFPRY	PND SVYANWM	LSPSAAKLMD	TFDS			1140

blue: RET sequence expressed in fusionprotein Red: variant in fusionprotein

¹NCBI/Protein accession number NP_066124.1

Recombinant Proteins