

# Certificate of Analysis

## CCDC6-RET

CCDC6-RET fusion protein variant a

Recombinant Human Active Protein Kinase

HGNC Symbol: n/a

Synonyms: CCDC6-RETA

Product No.: 1417-0000-1

Lot: 003

**Description:** Full length human CCDC6-RET fusion protein, amino acids Met1-Ser503 (as in NCBI/Protein entry BAM36435.1), N-terminal GST-HIS<sub>6</sub> fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

**Product identity:** CCDC6-RET Lot 003, was confirmed as a CCDC6 and RET fusionprotein by mass spectroscopy LC-ESI-MS/MS

**Theoretical MW<sub>Fusion Protein</sub>:** 84,394 Da

**Expression:** Baculovirus infected Sf9 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

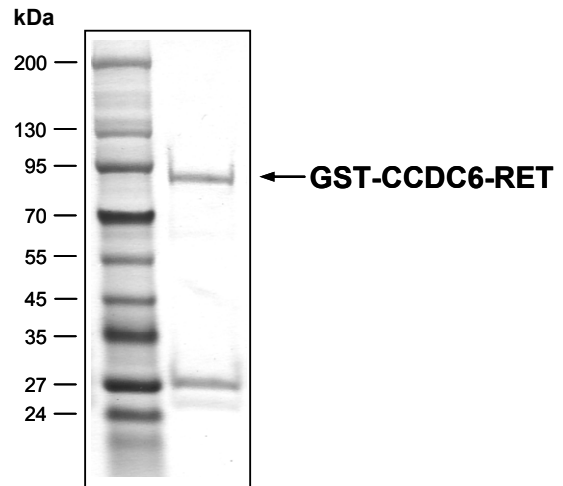
**Storage temperature:** -80°C  
Avoid repeated freeze-thaw cycles!

**Protein concentration:** 0.178 µg/µl  
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

### Biochemical Parameters:

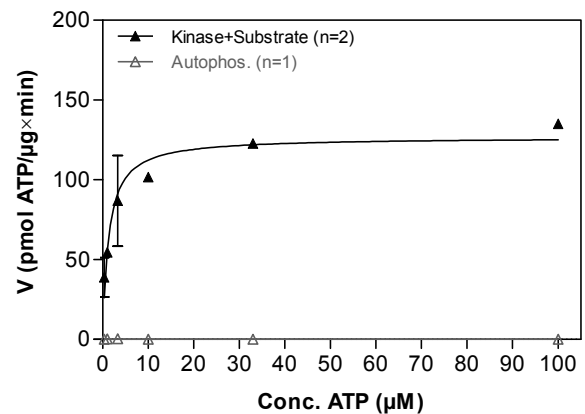
Specific kinase activity (P<sub>i</sub> transfer): 127 pmol/µg×min  
ATP-K<sub>M</sub>: 1.3 µM

### CCDC6-RET Lot 003: Coomassie stain



2.0 µg GST-CCDC6-RET

### CCDC6-RET Lot 003: Determination of V<sub>max</sub> and K<sub>M</sub> value for ATP



### Determination of K<sub>M</sub> value & Specific activity:

- Assay conditions:
  - 60 mM HEPES-NaOH, pH 7.5
  - 3 mM MgCl<sub>2</sub>
  - 3 mM MnCl<sub>2</sub>
  - 3 µM Na-orthovanadate
  - 1.2 mM DTT
  - 50 µg / ml PEG<sub>20,000</sub>
  - ATP (variable)
  - Substrate: TRK-C derived peptide R11-VYSTDYRFLNPS 40µg/ml
  - CCDC6-RET: 1.0 µg / ml
- Filter binding assay
  - MSFC membrane (Millipore)

**Additional assay technology:** CCDC6-RET Lot 003 was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from



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## CCDC6-RET

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CCDC6-RET Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSM	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPE	KMFKDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHHG	RDSLEVLFOG	240
241	PLAMGARGRM	ADSASESDTD	GAGGNSSSSA	AMQSSCSSTS	GGGGGGGGGG	GGGKSGGIVI	300
301	SPFRLEELTN	RLASLQQENK	VLKIELETYK	LKCKALQEEN	RDLRKASVTI	EDPKWEFPRK	360
361	NLVLGKTLGE	GEFGKVVKAT	AFHLKGRAGY	TTVAVKMLKE	NASPSELRLD	LSEFNVLKQV	420
421	NHPHVIKLYG	ACSQDGPLLL	IVEYAKYGS	RGFLRESRKV	GGYLGSGGS	RNSSSLDHPD	480
481	ERALTMGDLI	SFAWQISQGM	QYLAEMKLVH	RDLAARNILV	AEGRKMKISD	FGLSRDVYEE	540
541	DSYVKRSQGR	IPVKWMAIES	LFDHIYTTQS	DVWSFGVLLW	EIVTLGGNPY	PGIPPERLFN	600
601	LLKTGHRMER	PDNCSEEMYR	LMLQCWKQEP	DKRPVFADIS	KDLEKMMVKR	RDYDLAAS	660
661	PSDSLIIYDDG	LSEETPLVD	CNNAPLPRAL	PSTWIENKLY	GMSDPNWPGE	SPVPLTRADG	720
721	TNTGFPRYPN	DSVYANWMLS	PSAAKLMDTF	DS			780

1-218: GST Red: HIS6-tag Green: 3C blue:CCDC6-RET

CCDC6-RET <sup>1</sup> amino acid sequence							
1	MADSASESDT	DGAGGNSSSS	AAMQSSCSST	SGGGGGGGGG	GGGKSGGIV	ISPFRLEELT	60
61	NRLASLQQEN	KVLKIELETY	KLKCKALQEE	NRDLRKASVT	EDPKWEFPR	KNLVLGKTLG	120
121	EGEFGKVKA	TAFHLKGRAG	YTTVAVKMLK	ENASPSELRD	LLSEFNVLKQ	VNHPHVIKLY	180
181	GACSQDGPLL	LIVEYAKYGS	LRGFLRESRK	VGGYLGSGG	SRNSSSLDHP	DERALTMGDL	240
241	ISFAWQISQ	MQYLAEMKLV	HRDLAARNIL	VAEGRKMKIS	DFGLSRDVYE	EDSYVKRSQG	300
301	RIPVKWMAIE	SLFDHIYTTQ	SDVWSFGVLL	WEIVTLGGNP	YPGIPPERLF	NLLKTGHRME	360
361	RPDNCSEEMY	RLMLQCWKQE	PDKRPVFADI	SKDLEKMMVK	RDYDLAAS	TPSDSLIYDD	420
421	GLSEETPLV	DCNNAPLPR	LPSTWIENKL	YGMSDPNWP	ESPVPLTRAD	GTNTGFPRYP	480
481	NDSVYANWML	SPSAAKLMDT	FDS				540

blue: CCDC6-RET sequence expressed in fusionprotein E<sub>102</sub>: End of CCDC6 / start of RET ORF

<sup>1</sup>NCBI/Protein accession number BAM36435.1