

## Certificate of Analysis

### Ret (V804L), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-758, 14-758-K, 14-758M

Parent Lot # D8CN007U

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

**Product Description:** N-Terminal GST-tagged, recombinant, human Ret amino acids 658–end containing the V804L mutation. Expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose.

The V804L mutation has been identified in patients with familial medullary thyroid carcinoma (FMTC) (Lombardo F. et al., (2002), JCEM, 87, 1674-1680; Lesueur F. et al., (2005), JCEM, 90, 3454-3457).

Purity 78% by SDS-PAGE and Coomassie blue staining. MW = 79.2kDa.

**Specific Activity (Parent lot# D8CN007U):** 99U/mg, where one unit of Ret(V804L), active activity is defined as 1nmol phosphate incorporated into 100µM CHKtide (KKKVSRSGLYRSPSPENLNRP) per minute at 30°C with a final ATP concentration of 100µM.

**Formulation:** 1.578mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

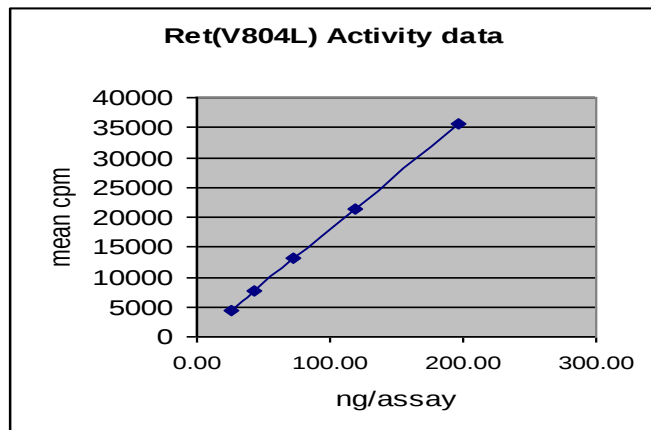
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**Handling Recommendations:** Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

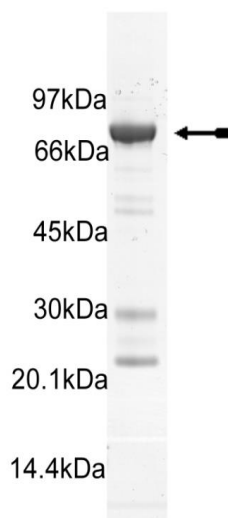
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 71–197ng of this lot of enzyme phosphorylated 100µM CHKtide (KKKVSRSGLYRSPSPENLNRP) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



**MS Tryptic Fingerprint:** Confirmed identity as Ret(V804L) with the translated sequence listed on page three.



**SDS-PAGE and Coomassie Stain:** Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of Ret (V804L), active.

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### Kinase Assay Protocol

#### Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **CHKtide (KKKVSRSGLYRSPSPENLNRPR):** Use at a final assay concentration of 100 $\mu$ M. Prepare a 1mM stock and add 2.5 $\mu$ l of stock per assay point.
3. **NaCl:** Use at a final assay concentration of 50mM. Make a 3M stock. Add 0.42 $\mu$ l of stock per assay point.
4. **Ret (V804L), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 71–197ng per assay point.
5. **[ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<sup>33</sup>P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

#### Assay Procedure (96 well plate format):

1. Add 5 $\mu$ l of 5 x reaction buffer to wells.
2. Add 2.5 $\mu$ l of (KKKVSRSGLYRSPSPENLNRPR).
3. Add **2.5 $\mu$ l (71–197ng) Ret(V804L), active.**
4. Add 0.42 $\mu$ l of 3M NaCl.
5. Add 4.58 $\mu$ l of dH<sub>2</sub>O.
6. Add 10 $\mu$ l of diluted [ $\gamma$ -<sup>33</sup>P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5 $\mu$ l 3% phosphoric acid.
9. Transfer a 10 $\mu$ l aliquot onto the appropriate area of a **P30 Filtermat**.
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 $\mu$ l of 30% phosphoric acid.

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## Ret (V804L) Sequence Information

|                         |                                                                        |
|-------------------------|------------------------------------------------------------------------|
| <b>Protein</b>          | Human Ret (658-end, V804L)                                             |
| <b>Tags</b>             | N-Terminal GST                                                         |
| <b>Native sequence</b>  | H237 of recombinant sequence is equivalent to H658 of native human Ret |
| <b>Accession number</b> | GenBank NM_000323                                                      |

### Recombinant Ret (V804L) amino acid sequence:

```

1  MSPILGYWKI  KGLVQPTRLL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMa  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121 DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181 KRIEAIQID   KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LEVLFQGPFE  KGLRRQHCHY
241 KFAHKPPISS  AEMTFRRPAQ  AFPVSYSSSG  ARRPSLDSME  NQVSVDFAFK  LEDPKWEFPR
301 KNLVLGKTLG  EGEFGKVVKA  TAFHLKGRAG  YTTVAVKMLK  ENASPELDRD  LLSEFNVLKQ
361 VNHPHVIKLY  GACSQDGPLL  LILEYAKYGS  LRGFLRESRK  VGPGYLGSGG  SRNSSSLDHP
421 DERALTMGDL  ISFAWQISQG  MQYLAEMKLV  HRDLAARNIL  VAEGRKMKIS  DFGLSRDVYE
481 EDSYVKRSQG  RIPVKWMAIE  SLFDHIYTTQ  SDVWSFGVLL  WEIVTLGGNP  YPGIPPERLF
541 NLLKTGHRME  RPDNCSEEMY  RLMLQCWKQE  PDKRPVFADI  SKDLEKMMVK  RRDYDLAAS
601 TPSDSLIIYD  GLSEETPLV   DCNNAPLPRA  LPSTWIENKL  YGMSDPNWPG  ESPVPLTRAD
661 GTNTGFPRYP  NDSVYANWML  SPSAAKLMDT  FDS

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### Recombinant Ret (V804L) nucleotide sequence:

```

1  atgtccccta  tactaggtta  ttggaataat  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatatc  ttgaagaaaa  atatgaagag  catttgatat  agcgcatga  aggtgataaa
121 tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatatgat
181 ggtgatgtta  aattaacaca  gtctatggcc  atcatacggt  atatacgtga  caagcacaa
241 atgttgggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcggttttg
301 gatattagat  acggtgtttc  gagaattgca  tatagtaaag  actttgaaac  tctcaaagtt
361 gattttctta  gcaagctacc  tgaaatgctg  aaaatgttcg  aagatcggtt  atgtcataaa
421 acatatttaa  atggtgatca  tgtaacccat  cctgacttca  tggtgtatga  cgctcttgat
481 gttgttttat  acatggaccc  aatgtgcctg  gatgcgttcc  caaaattagt  ttgttttaaa
541 aaacgtattg  aagctatccc  acaaattgat  aagtacttga  aatccagcaa  gtatatagca
601 tggcctttgc  agggctggca  agccacgttt  ggtggtggcg  accatcctcc  aaaatcggat
661 ctggaagtgc  tgttccaggg  gcccgaaatt  aaaggcctac  gtcgacaaca  ctgctaccac
721 aagtttgccc  acaagccacc  catctcctca  gctgagatga  ccttcaggag  gccgcggcag
781 gccttcccg  tcagctactc  ctcttcgggt  gccgcgggc  cctcgtgga  ctccatggag
841 aaccaggtct  ccgtggatgc  cttcaagatc  ctggaggatc  caaagtggga  attccctcgg
901 aagaacttgg  ttcttgaaa  aactctagga  taaggcgaat  ttgaaaagt  ggtcaaggca
961 aggccttcc  atctgaaagg  cagagcaggg  tacaccacgg  tggcgtgaa  gatgctgaaa
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1201 gtggggcctg  gctacctggg  cagtggaggc  agccgcaact  ccagctccct  ggaccaccg
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1501 tccctttttg  atcatatcta  caccacgcaa  agtgatgtat  ggtcttttgg  tgtcctgctg
1561 tgggagatcg  tgaccctagg  gggaaacccc  tatcctggga  ttctcctga  gcggtctctc
1621 aaccttctga  agaccggcca  ccggatggag  aggccagaca  actgcagcga  ggagatgtac
1681 cgctgatgc  tgcaatgctg  gaagcaggag  ccggacaaaa  ggccggtgtt  tgcggacatc
1741 agcaaagacc  tggagaagat  gatggttaag  aggagagact  acttggaact  tgcggcgtcc

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```
1801 actccatctg actccctgat ttatgacgac ggccctctcag aggaggagac accgctggtg
1861 gactgtaata atgccccctt cctcgcagcc ctcccttcca catggattga aaacaaactc
1921 tatggcatgt cagacccgaa ctggcctgga gagagtcctg taccactcac gagagctgat
1981 ggactaaca ctgggtttcc aagatatcca aatgatagtg tatatgctaa ctggatgctt
2041 tcaccctcag cggcaaaatt aatggacacg ttgatagtt aa
```

Reviewed and approved by site quality representative.

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