

Certificate of Analysis

Ret (V804M), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-760, 14-760-K, 14-760M

Parent Lot # D8PN059U

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 V804M mutation. Expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose.

The V804M mutation has been identified in patients with familial medullary thyroid carcinoma (FMTC) (Fink et al, (1996), Int. J. Cancer, 69, 312-316; Lesueur F. et al, (2005), JCEM, 90, 3454-3457).

Purity 81% by SDS-PAGE and Coomassie blue staining. MW = 79.2kDa.

Formulation: 1.125mg/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.

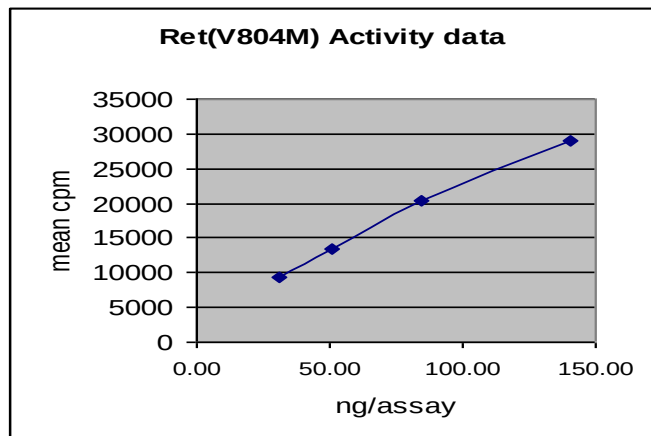
Specific Activity (Parent lot# D8PN059U): 131U/mg, where one unit of Ret (V804M), active activity is defined as 1nmol phosphate incorporated into 100µM CHKtide (KKKVSRSGLYRSPSPENLNRP) per minute at 30°C with a final ATP concentration of 100µM.

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.

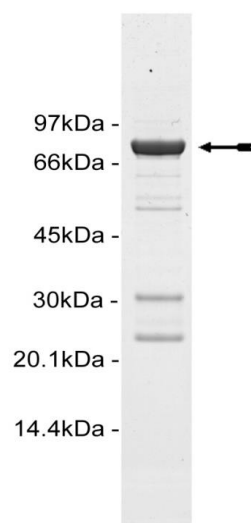
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 31–141ng 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 product identity as Ret (V804M) 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 (V804M), 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 μ M. Prepare a 1mM stock and add 2.5 μ l of stock per assay point.
3. **NaCl:** Use at a final assay concentration of 100mM. Make a 3M stock. Add 0.833 μ l of stock per assay point.
4. **Ret (V804M), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 31–141ng per assay point.
5. **[γ -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer to wells.
2. Add 2.5 μ l of (KKKVSRSGLYRSPSPENLNRPR).
3. Add **2.5 μ l (31–141ng) Ret (V804M), active.**
4. Add 0.833 μ l of 3M NaCl
5. Add 4.167 μ l of dH₂O.
6. Add 10 μ l of diluted [γ -³³P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5 μ l 3% phosphoric acid.
9. Transfer a 10 μ 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 μ l of 30% phosphoric acid.

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Ret (V804M) Sequence Information

<u>Protein</u>	Human Ret (658-end, V804M)
<u>Tags</u>	N-Terminal GST
<u>Native sequence</u>	H237 of recombinant sequence is equivalent to H658 of native human Ret
<u>Accession number</u>	GenBank NM_000323

Recombinant Ret (V804M) amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSM  IIRYIADKH  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121 DFLSKLPEML  KMFEDRLCH  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181 KRIEAI PQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LEVLFQGP EF  KGLRRQHCHYH
241 KFAHKPPISS  AEMTFRRPAQ  AFPVSYSSSG  ARRPSLDSME  NQVSVD AFKI  LEDPKWEFPR
301 KNLVLGKTLG  EGEFGKV VKA  TAFHLKGRAG  YTTVAVKMLK  ENASPS ELRD  LLSEFNVLKQ
361 VNHPHVIKLY  GACSQDGPLL  LIMEYAKYGS  LRGFLRESRK  VGPGLGSGG  SRNSSSLDHP
421 DERALTMGDL  ISFAWQISQG  MQYLAEMKLV  HRDLAARNIL  VAEGRKMKIS  DFGLSRDVYE
481 EDSYVKRSQG  RIPVKWMAIE  SLFDHIYTTQ  SDVWSFGVLL  WEIVTLGGNP  YPGIPPERLF
541 NLLKTGHRME  RPDNCSEEMY  RLMLQCWKQE  PDKRPVFADI  SKDLEKMMVK  RRDYDLAAS
601 TPSDSL IYDD  GLSEETPLV  DCNNAPLPRA  LPSTWIENKL  YGMSDPN WPG  ESPVPLTRAD
661 GTNTGFPRYP  NDSVYANWML  SPSAAKLMDT  FDS

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Recombinant Ret (V804M) nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatatc  ttgaagaaaa  atatgaagag  catttgatg  agcgcatga  aggtgataaa
121 tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatatgat
181 ggtgatgtta  aattaacaca  gtctatggcc  atcatagct  atatactga  caagcacaac
241 atgttgggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcggttttg
301 gatattagat  acggtgtttc  gagaattgca  tatagtaaag  actttgaaac  tctcaaagtt
361 gattttctta  gcaagctacc  tgaaatgctg  aaaatgttcg  aagatcgttt  atgtcataaa
421 acatatttaa  atggtgatca  tgtaacccat  cctgacttca  tggtgatga  cgctcttgat
481 gttgttttat  acatggaccc  aatgtgcctg  gatgcgttcc  caaaattagt  ttgttttaaa
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601 tggcctttgc  agggctggca  agccacgttt  ggtggtggcg  accatcctcc  aaaatcggat
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1621 aaccttctga  agaccggcca  ccggatggag  aggccagaca  actgcagcga  ggagatgtac
1681 cgctgatgc  tgcaatgctg  gaagcaggag  ccggacaaaa  ggccggtgtt  tgccgacatc
1741 agcaaagacc  tggagaagat  gatggttaag  aggagagact  acttggaact  tgcggcgtcc

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