

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.

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

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.

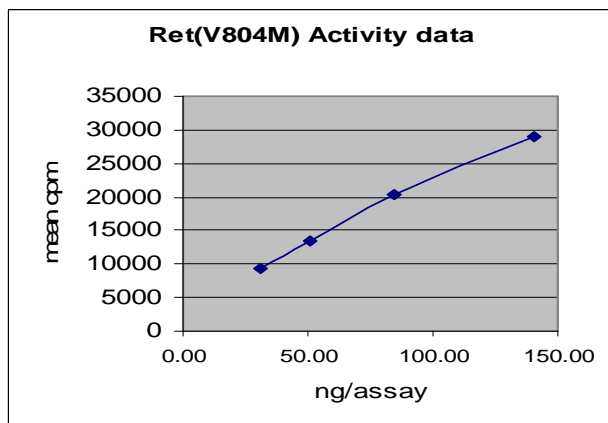
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

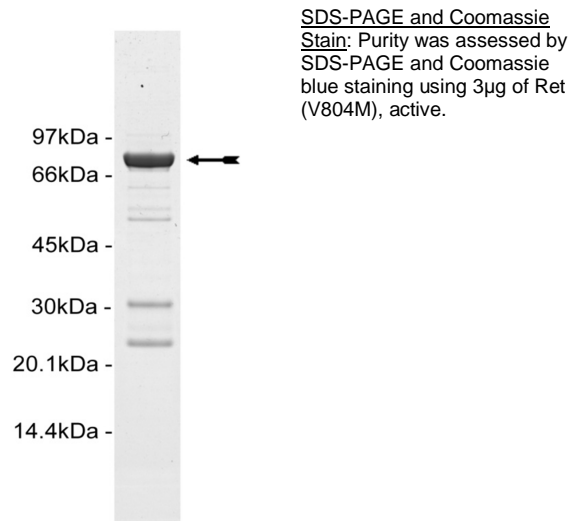
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 31–141ng of this lot of enzyme phosphorylated 100µM CHKtide (KKKVSRSGLYRSPSPENLNRPR) 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.



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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 KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQSMS IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIQID KYLKSSKYIA WPLQGQATF GGGDHPPKSD LEVLFQGPPEF KGLRRQHCHYH
241 KFAHKPPISS AEMTFRRPAQ AFPVSYSSSG ARRPSLDSME NQVSVDAFKI LEDPKWEFPR
301 KNLVLGKTLG EGEFGKVVKA TAFHLKGRAG YTTVAVKMLK ENASPSSELRD LLSEFNVLKQ
361 VNHPHVIKLY GACSQDGPLL LIMEYAKYGS LRGFRESRK VGPGYLGS GG SRNSSSLDHP
421 DERALTMGDL ISFAWQISQG MQYLAEMKLV HRDLAARNIL VAEGRKMKIS DFGLSRDVYE
481 EDSYVKRSQG RIPVKWMAIE SLFDHIYTTQ SDVVSFGVLL WEIVTLGGNP YPGIPPERLF
541 NLLKTGHRME RPDNCSEEMY RLMLQCWKQE PDKRPVFADI SKDLEKMMVK RRDYLDLAAS
601 TPSDSLIIYDD GLSEETPLV DCNNAPL PRA LPSTWIENKL YGMSDPNWP G 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 togacttctt
61 ttggaatata ttgaagaaaa atatgaagag catttgatg agcgcgatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggtttg gagtttcca atcttcctta ttatatgat
181 ggtgatgta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaag agcggttttg
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481 gttgttttat acatggacc aatgtgcctg gatgcgttcc caaaattagt ttgttttaa
541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc agggctggca agccacgttt ggtgggtggc accatcctcc aaaatcggat
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721 aagtttgccc acaagccacc catctcctca gctgagatga ccttccggag gcccgccag
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1321 atgcagatc tggccgagat gaagctcggt catcgggact tggcagccag aaacatcctg
1381 gttagctgagg ggcggaagat gaagatttcg gatttcggct tgtcccgaga tgtttatgaa
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1501 tccctttttg atcatatcta caccacgcaa agtgatgat ggtctttttg tgtcctgctg
1561 tgggagatcg tgaccctagg gggaaacccc tatcctggga ttcctcctga gcggctcttc
  
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1621 aaccttctga agaccggcca cgggatggag aggccagaca actgcagcga ggagatgtac
1681 cgctgatgc tgcaatgctg gaagcaggag cggacaaaa ggccggtgtt tgcggacatc
1741 agcaaagacc tggagaagat gatggttaag aggagagact acttggacct tgcggcgtcc
1801 actccatctg actccctgat ttatgacgac ggctctcag aggaggagac accgctgggtg
1861 gactgtaata atgccccctt cctcagagcc ctcccttcca catggattga aaacaaactc
1921 tatggcatgt cagaccgaa ctggcctgga gagagtctg taccactcac gagagctgat
1981 ggcactaaca ctgggtttcc aagatatcca aatgatagtg tatatgctaa ctggatgctt
2041 tcaccctcag cggcaaaatt aatggacacg tttgatagtt aa
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