eurofins | Discovery Services

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) activity is defined as 1nmol phosphate incorporated into 100µM CHKtide (KKKVSRSGLYRSPSMPENLNRPR) 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.

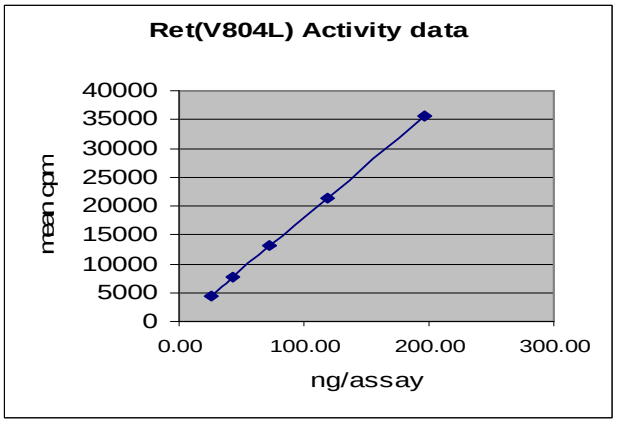
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

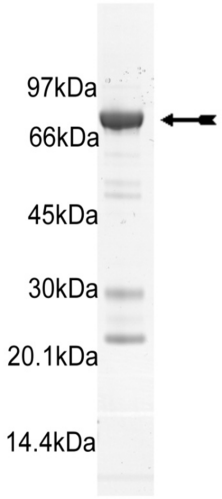
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 71–197ng of this lot of enzyme phosphorylated 100µM CHKtide (KKKVSRSGLYRSPSMPENLNRPR) 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.

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

eurofins | Discovery Services

Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **CHKtide (KKKVSRSGLYRSPSMPENLNRPR):** Use at a final assay concentration of 100µM. Prepare a 1mM stock and add 2.5µl of stock per assay point.
3. **NaCl:** Use at a final assay concentration of 50mM. Make a 3M stock. Add 0.42µl 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. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer to wells.
2. Add 2.5µl of **(KKKVSRSGLYRSPSMPENLNRPR)**.
3. Add **2.5µl (71–197ng) Ret(V804L), active**.
4. Add 0.42µl of 3M NaCl.
5. Add 4.58µl of dH_2O.
6. Add 10µl of diluted [γ-^{33}P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl 3% phosphoric acid.
9. Transfer a 10µl 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 1µl of 30% phosphoric acid.

eurofins | Discovery Services

Certificate of Analysis

Ret (V804L) Sequence Information

Protein	Human Ret (658-end, V804L)
Tags	*N*-Terminal GST
Native sequence	H237 of recombinant sequence is equivalent to H658 of native human Ret
Accession number	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 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPEF KGLRRQHCYH
241 KFAHKPPISS AEMTFRRPAQ AFPVSYSSSG ARRPSLDSME NQVSVDAFKI LEDPKWEFPR
301 KNLVLGKTLG EGEFGKVVKA TAFHLKGRAG YTTVAVKMLK ENASPSELRD 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 RRDYLDLAAS
601 TPSDSLIYDD GLSEEETPLV DCNNAPLPRA LPSTWIENKL YGMSDPNWPG ESPVPLTRAD
661 GTNTGFPRYP NDSVYANWML SPSAAKLMDT FDS
```

Recombinant Ret (V804L) nucleotide sequence:

```
   1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaacccac tcgacttctt
  61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
 121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
 181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga 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 tgttgtatga cgctcttgat
 481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
 541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
 601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
 661 ctggaagttc tgttccaggg gcccgaattc aaaggcctac gtcgacaaca ctgctaccac
 721 aagtttgccc acaagccacc catctcctca gctgagatga ccttccggag gcccgcccag
 781 gccttcccgg tcagctactc ctcttccggt gcccgccggc cctcgctgga ctccatggag
 841 aaccaggtct ccgtggatgc cttcaagatc ctggaggatc caaagtggga attccctcgg
 901 aagaacttgg ttcttggaaa aactctagga gaaggcgaat ttggaaaagt ggtcaaggca
 961 acggccttcc atctgaaagg cagagcaggg tacaccacgg tggccgtgaa gatgctgaaa
1021 gagaacgcct ccccgagtga gcttcgagac ctgctgtcag agttcaacgt cctgaagcag
1081 gtcaaccacc cacatgtcat caaattgtat ggggcctgca gccaggatgg cccgctcctc
1141 ctcatcttgg agtacgccaa atacggctcc ctgcggggct tcctccgcga gagccgcaaa
1201 gtggggcctg gctacctggg cagtggaggc agccgcaact ccagctccct ggaccacccg
1261 gatgagcggg ccctcaccat gggcgacctc atctcatttg cctggcagat ctcacagggg
1321 atgcagtatc tggccgagat gaagctcgtt catcgggact tggcagccag aaacatcctg
1381 gtagctgagg ggcggaagat gaagatttcg gatttcggct tgtcccgaga tgtttatgaa
1441 gaggattcct acgtgaagag gagccagggt cggattccag ttaaatggat ggcaattgaa
1501 tccctttttg atcatatcta caccacgcaa agtgatgtat ggtcttttgg tgtcctgctg
1561 tgggagatcg tgaccctagg gggaaacccc tatcctggga ttcctcctga gcggctcttc
```

eurofins | Discovery Services

Certificate of Analysis

```
1621 aaccttctga agaccggcca ccggatggag aggccagaca actgcagcga ggagatgtac
1681 cgcctgatgc tgcaatgctg gaagcaggag ccggacaaaa ggccggtgtt tgcggacatc
1741 agcaaagacc tggagaagat gatggttaag aggagagact acttggacct tgcggcgtcc
1801 actccatctg actccctgat ttatgacgac ggcctctcag aggaggagac accgctggtg
1861 gactgtaata atgcccccct ccctcgagcc ctcccttcca catggattga aaacaaactc
1921 tatggcatgt cagacccgaa ctggcctgga gagagtcctg taccactcac gagagctgat
1981 ggcactaaca ctgggtttcc aagatatcca aatgatagtg tatatgctaa ctggatgctt
2041 tcaccctcag cggcaaaatt aatggacacg tttgatagtt aa
```

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 **Eurofins Pharma Discovery Services UK Limited** is an independent member of Eurofins Discovery Services.