

Certificate of Analysis

Ret, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-570, 14-570-K, 14-570M

Parent Lot # 1654890

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, expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose. Purity 79% by SDS-PAGE and Coomassie blue staining. MW = 79.2kDa.

Specific Activity (Parent lot# 1654890): 1260U/mg, where one unit of Ret, active activity is defined as 1nmol phosphate incorporated into 250μM (KKKSPGEYVNIEFG) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 1.023mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

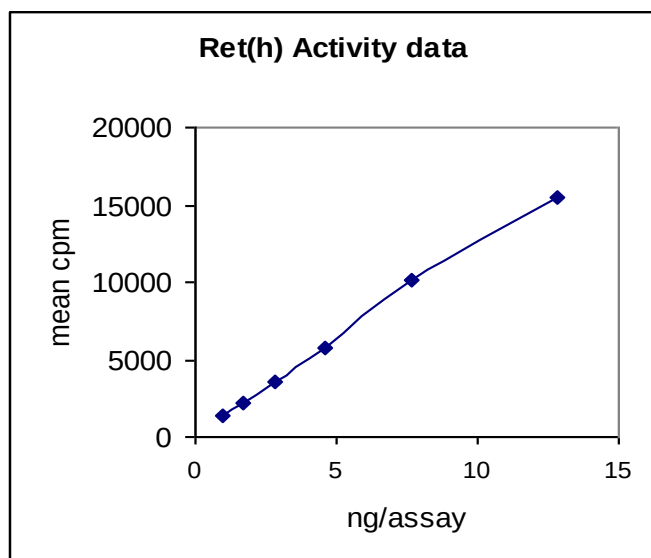
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

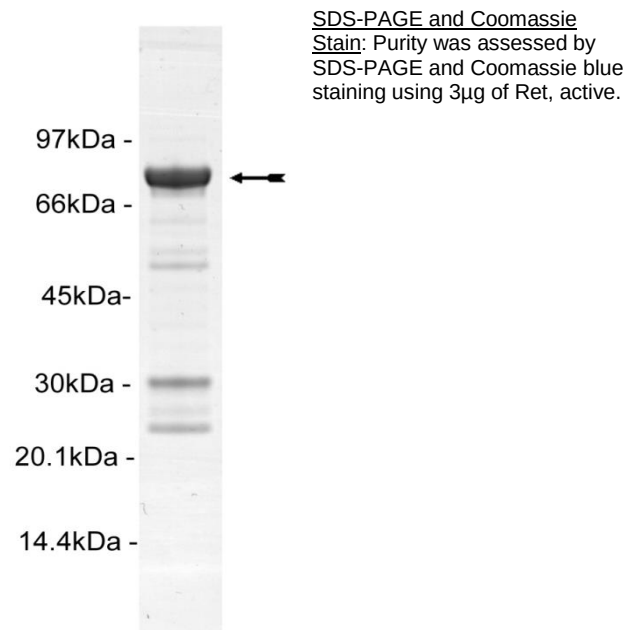
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1.0–12.8ng of this lot of enzyme phosphorylated 250μM (KKKSPGEYVNIEFG) 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 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. **(KKKSPGEYVNIEFG):** Use at a final assay concentration of 250µM. Prepare a 2.5mM stock and add 2.5µl of stock per assay point.
3. **Ret, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.0–12.8ng per assay point.
4. **[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 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **(KKKSPGEYVNIEFG)**.
3. Add **2.5µl (1.0–12.8ng) Ret, active**.
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. 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.

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Ret Sequence Information

<u>Protein</u>	Human Ret
<u>Tags</u>	N-Terminal GST
<u>Native sequence</u>	H237 of the recombinant protein is equivalent to H658 of human Ret
<u>Accession number</u>	GenBank NM_000323

Recombinant Ret amino acid sequence:

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1 MSPILGYWKI KGLVQPTRL L LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFGQPEF KGLRRQHCYH
241 KFAHKPIISS AEMTFRRPAQ AFPVSYSSSG ARRPSLDSME NQVSVDAFKI LEDPKWEFPR
301 KNLVLGKTLG EGEFGKVKA TAFHLKGRAG YTTVAVKMLK ENASPSELRD LLSEFNVLKQ
361 VNHPHVIKLY GACSQDGPLL LIVEYAKYGS LRGFLRESRK VGPYGLSGG SRNSSSLDHP
421 DERALTMGDL ISFAWQISQG MQYLAEMKLV HRDLAARNIL VAEGRKMKIS DFGLSRDVYE
481 EDSYVKRSQG RIPVKWMAIE SLFDHIYTTQ SDVWSFGVLL WEIVTLGGNP YPGIPPERLF
541 NLLKTGHRME RPDNCSEEMY RLMLQCWKQE PDKRPVFADI SKDLEKMMVK RRDYDLAAS
601 TPSDSLIIYDD GLSEETPLV DCNNAPLPRA LPSTWIENKL YGMSDPNWPGE ESPVPLTRAD
661 GTNTGFPRYP NDSVYANWML SPSAAKLMDT FDS

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Recombinant Ret nucleotide sequence:

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1 atgtccccta tactagggtta ttggaaaatt aagggccttg tgcaaccacac tcgacttctt
61 ttggaatatac ttgaagaaaa atatgaagag catttgatatg agcgcatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatatgtat
181 ggtgatgtta aattaacaca gtctatggcc atcatagctt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcgggtttg
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1621 aaccttctga agaccggcca ccgatggag aggcagaca actgcagcga ggagatgtac

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1681 cgcctgatgc tgcaatgctg gaagcaggag ccggacaaaa ggccggtggtt tgcggacatc
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1861 gactgtaata atgccccctt ccctcgagcc ctcccttcca catggattga aaacaaactc
1921 tatggcatgt cagacccgaa ctggcctgga gagagtcctg taccactcac gagagctgat
1981 ggcactaaca ctgggtttcc aagatatcca aatgatatgt tatatgctaa ctggatgctt
2041 tcaccctcag cggcaaaatt aatggacacg tttgatagtt aa
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