

Certificate of Analysis

Flt3, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-500, 14-500-K, 14-500M

Parent Lot # WAA0196

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 Flt3, amino acids 564–end, expressed by baculovirus in Sf21 insect cells. Purified using glutathione-Sepharose. Purity 95.3% by SDS-PAGE and Coomassie blue staining. MW = 77.4kDa.

Specific Activity (Parent lot# WAA0196): 152U/mg, where one unit of Flt3 activity is defined as 1nmol phosphate incorporated into 50µM Abltide (EAIYAAPFAKKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.198mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 270mM sucrose, 0.03% Brij-35, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

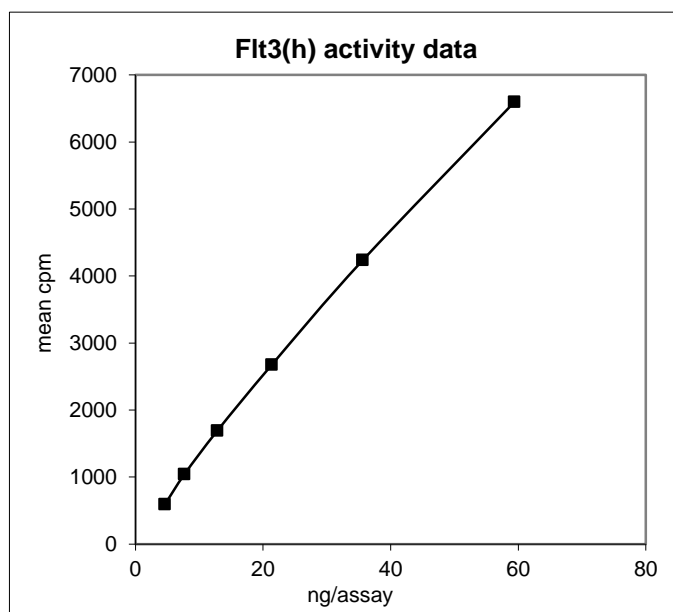
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

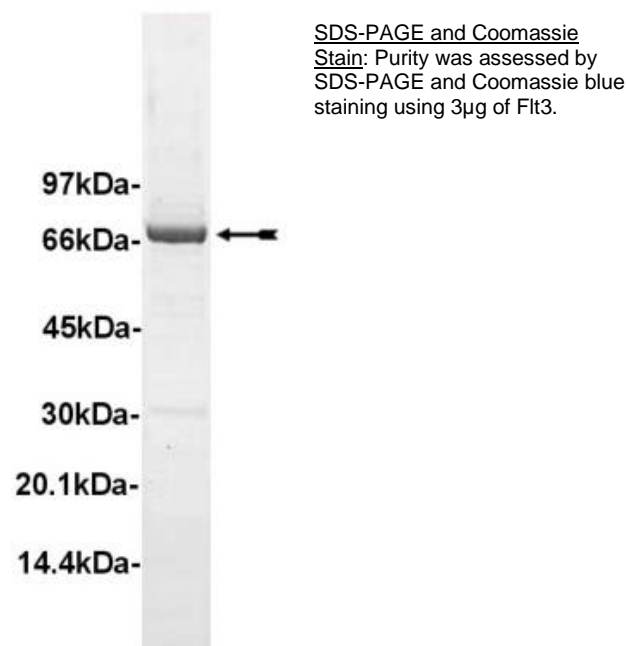
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 4.6–59.4ng of this lot of enzyme phosphorylated 50µM Abltide 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 Flt3 with the translated sequence listed on page three.



Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS pH7.0, 1mM EDTA.
2. **Abtide (EAIYAAPFAKKK):** Use at a final assay concentration of 50 μ M. Prepare a 500 μ M stock. Add 2.5 μ l of stock per assay point.
3. **Flt3, active:** Dilute with 20mM MOPS pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 4.6–59.4ng 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 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **abtide**.
3. Add **2.5 μ l (4.6–59.4ng) Flt3, active**.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l 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 1 μ l of 30% phosphoric acid.

Certificate of Analysis

Flt3 Sequence Information

<u>Protein</u>	Human Flt3
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	H237 of the recombinant protein is equivalent to H546 of human Flt3
<u>Accession number</u>	GenBank NM_004119

Recombinant Flt3 amino acid sequence:

```

1 MSPILGYWKI KGLVQPTRL L LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQ SMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLP EML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KR IEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGP EF KGLRRRHKYK
241 KQFRYESQLQ MVQVTGSSDN EYFYVDFREY EYDLKWEFPR ENLEFGKVLG SGAFGKVMNA
301 TAYGISKTGV SIQVAVKMLK EKADSSEREA LMSELKMMTQ LGSHENIVNL LGACTLSGPI
361 YLIFEYCCYG DLLNYLRSKR EKFHRTWTEI FKEHNFSFYP TFQSHPNSSM PGSREVQIHP
421 DSDQISGLHG NSFHSEDEIE YENQKRLEEE EDLNVLT FED LLCFAYQVAK GMEFLEFKSC
481 VHRDLAARNV LVTHGKVVKI CDFGLARDIM SDSNYVVRGN ARLPVKWMAP ESLFEGIYTI
541 KSDVWSYGIL LWEIFSLGVN PYPGIPVDAN FYKLIQNGFK MDQPFYATEE IYIIMQSCWA
601 FDSRKRPSPF NLTSFLGCQL ADAEEAMYQN VDGRVSECPH TYQNRPF SR EMDLGLLSPQ
661 AQVEDS

```

Recombinant Flt3 nucleotide sequence:

```

1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatadc ttgaagaaaa atatgaagag catttgatg agcgcgatga aggtgataaa
121 tggcgaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
181 ggtgatgtta aattaacaca gtctatggcc atcatagctt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
361 gattttctta gcaagctacc tgaatgctg aaaatgctc aagatcgttt atgtcataaa
421 acatatttaa atggtgatca tgtaaccat cctgacttca tgttgatga cgctcttgat
481 gttgttttat acatggacc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gatatatgca
601 tggcctttgc agggctggca agccacgttt ggtgggtggcg accatcctcc aaaatcggat
661 ctggaagttc tgttcaggg gcccgaattc aaaggcctac gtcgacgaca caagtacaaa
721 aagcaattta ggatgaaag ccagctacag atggtacagg tgaccggctc ctcagataat
781 gactacttct acgttgattt cagagaatat gaatatgac tcaaatggga gtttccaaga
841 gaaaatttag agtttgggaa ggtactagga tcagggtgctt ttggaaaagt gatgaacgca
901 acagcttatg gaattagcaa aacaggagtc tcaatccagg ttgccgtcaa aatgctgaaa
961 gaaaaagcag acagctctga aagagaggca ctcatgtcag aactcaagat gatgaccag
1021 ctgggaagcc acgagaatat tgtgaacctg ctgggggctg gcacactgtc aggaccaatt
1081 tacttgattt ttgaatactg ttgctatggt gatcttctca actatctaag aagtaaaaga
1141 gaaaaatttc acaggacttg gacagagatt ttcaaggaa acaatttcag tttttacccc
1201 actttccaat cacatccaaa ttccagcatg cctggttcaa gagaagttca gatacaccg
1261 gactcggatc aaatctcagg gcttcatggg aattcatttc actctgaaga tgaaattgaa
1321 tatgaaaacc aaaaaaggct ggaagaagag gaggacttga atgtgcttac atttgaagat
1381 cttctttgct ttgcatatca agttgcaaaa ggaatggaat ttctggaatt taagtcgtgt
1441 gttcacagag acctggccgc caggaacgtg cttgtcacc acgggaaagt ggtgaagata
1501 tgtgactttg gattggctcg agatatcatg agtgattcca actatgttgt caggggcaat
1561 gccgtctgc ctgtaaaatg gatggcccc gaaagcctgt ttgaaggcat ctacaccatt
1621 aagagtgatg tctggtcata tggaatatta ctgtgggaaa tcttctcact tgggtgtaat

```

Certificate of Analysis

```
1681 ccttacctg gcattccggt tgatgctaac ttctacaaac tgattcaaaa tggatttaaa
1741 atggatcagc cattttatgc tacagaagaa atatacatta taatgcaatc ctgctgggct
1801 ttgactcaa ggaaacggcc atccttcctt aatttgactt cgtttttagg atgtcagctg
1861 gcagatgcag aagaagcgat gtatcagaat gtggatggcc gtgtttcgga atgtcctcac
1921 acctacaaa acaggcgacc tttcagcaga gagatggatt tggggctact ctctccgcag
1981 gctcaggtcg aagattcgta g
```

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.