

Certificate of Analysis

Flt3 (D835Y), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-610, 14-610-K, 14-610M

Parent Lot # 33205U

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 Flt3 amino acids 564–end, containing the mutation D835Y. This mutation is thought to produce a constitutively active form of Flt3 and has been shown to be associated with acute myeloid leukaemia. It is expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose. Purity 26% by SDS-PAGE and Coomassie blue staining. MW = 77.4kDa.

Specific Activity (Parent lot# 33205U): 166U/mg, where one unit of Flt3 (D835Y, active) activity is defined as 1nmol phosphate incorporated into 50µM Abltide (EAIYAAPFAKKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.732mg/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.

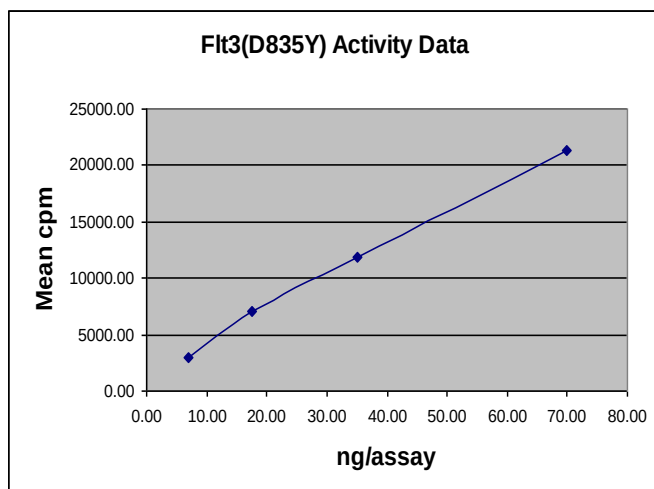
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

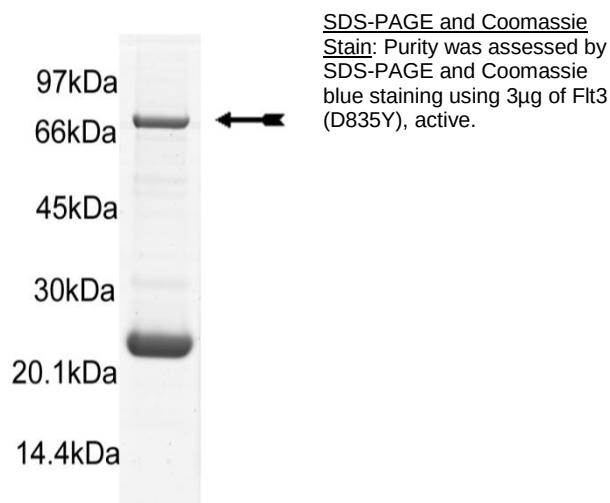
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 7–70ng 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 identity as Flt3 (D835Y) with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **Assay Dilution Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Abltide (EAIYAAPFAKKK):** Use at a final assay concentration of 50 μ M. Prepare a 500 μ M stock and add 2.5 μ l of stock per assay point.
3. **Flt3 (D835Y), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 7–70ng 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 **Abltide**.
3. Add **2.5 μ l (7–70ng) Flt3 (D835Y), 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.

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Flt3 (D835Y) Sequence Information

<u>Protein</u>	Human Flt3 (D835Y)
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	H237 of recombinant sequence is equivalent to H564 of native human Flt3
<u>Accession number</u>	GeneBank NM_004119

Recombinant Flt3 (D835Y) amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121 DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181 KRIEAIPQID  KYLKSSKYIA  WPLQGQWATF  GGGDHPPKSD  LEVLFQGPEF  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  EDLNVLTTFE  LLCFAYQVAK  GMEFLEFKSC
481 VHRDLAARNV  LVTHGKVVKI  CDFGLARYIM  SDSNYVVRGN  ARLPVKWMAP  ESLFEGIYTI
541 KSDVWSYGIL  LWEIFSLGVN  PYPGIPVDAN  FYKLIQNGFK  MDQPFYATEE  IYIIMQSCWA
601 FDSRKRPSPF  NLTSFLGCQL  ADAEEAMYQN  VDGRVSECPH  TYQNRPFPSR  EMDLGLLSPQ
661 AQVEDS

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Recombinant Flt3 (D835Y) nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatatc  ttgaagaaaa  atatgaagag  catttgatat  agcgcatga  aggtgataaa
121 tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatatgat
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361 gattttctta  gcaagctacc  tgaaatgctg  aaaatgttcg  aagatcgttt  atgtcataaa
421 acatatttaa  atggtgatca  tgtaacccat  cctgacttca  tggtgtatga  cgctcttgat
481 gttgttttat  acatggaccc  aatgtgcctg  gatgcgttcc  caaaattagt  ttgttttaaa
541 aaacgtattg  aagctatccc  acaaattgat  aagtacttga  aatccagcaa  gtatatagca
601 tggcctttgc  agggctggca  agccacgttt  ggtggtggcg  accatcctcc  aaaatcggat
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721 aagcaattta  ggtatgaaag  ccagctacag  atggtacagg  tgaccggctc  ctgagataat
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841 gaaaatttag  agtttgggaa  ggtactagga  tcagggtgct  ttggaaaagt  gatgaacgca
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1201 actttccaat  cacatccaaa  ttccagcatg  cctggttcaa  gagaagttca  gatacacccg
1261 gactcggatc  aaatctcagg  gcttcatggg  aattcatttc  actctgaaga  tgaaattgaa
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1381 cttctttgct  ttgcatatca  agttgccaaa  ggaatggaat  ttctggaatt  taagtcgtgt
1441 gttcacagag  acctggccgc  caggaaagtg  cttgtcacc  acgggaaagt  ggtgaagata
1501 tgtgactttg  gattggctcg  atatatcatg  agtgattcca  actatgttgt  caggggcaat

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1561 gcccgctctgc ctgtaaaatg gatggccccc gaaagcctgt ttgaaggcat ctacaccatt
1621 aagagtgatg tctggtcata tggaatatta ctgtgggaaa tcttctcact tgggtgtgaat
1681 ccttaccctg gcattccggt tgatgctaac ttctacaaac tgattcaaaa tggattttaa
1741 atggatcagc cattttatgc tacagaagaa atatacatta taatgcaatc ctgctgggct
1801 tttgactcaa ggaaacggcc atccttcctt aatttgactt cgtttttagg atgtcagctg
1861 gcagatgcag aagaagcgat gtatcagaat gtggatggcc gtgtttcgga atgtcctcac
1921 acctaccaa acaggcgacc ttccagcaga gagatggatt tggggctact ctctccgcag
1981 gctcaggtcg aagattcgta g
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