

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.

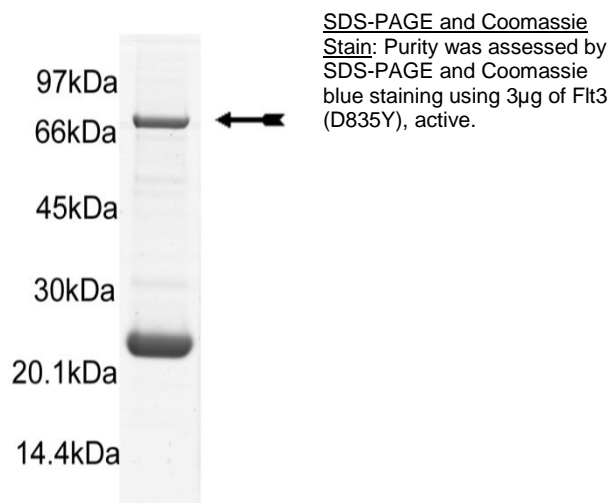
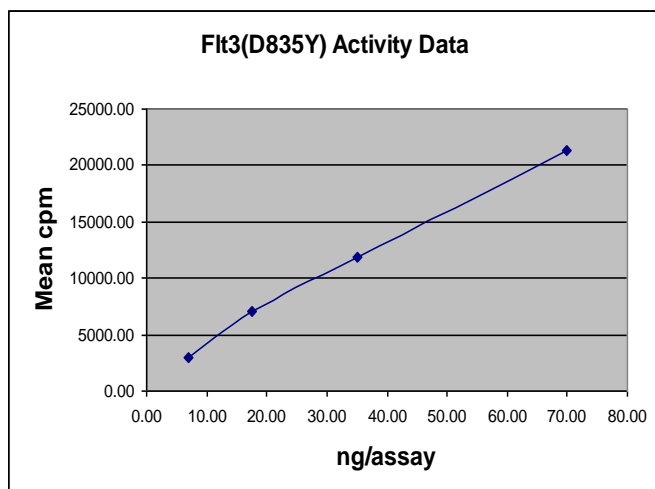
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

**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  KGLVQPTRLL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLPPEML  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  FDSRKRPSFP  NLTSFLGCQL  ADAEAMYQN  VDGRVSECPH  TYQNRPPFSR  EMDLGLLSPQ
661  AQVEDS

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Recombinant Flt3 (D835Y) nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatata  ttgaagaaaa  atatgaagag  catttgtatg  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatattgat
181  ggtgatgtta  aattaacaca  gtctatggcc  atcatacggt  atatagctga  caagcacaac
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481  gttgttttat  acatggacc  aatgtgctg  gatgcttcc  caaaattagt  ttgttttaa
541  aaacgtattg  aagctatccc  acaaattgat  aagtacttga  aatccagcaa  gtatatagca
601  tggcctttgc  agggctggca  agccacgttt  ggtggtggcg  accatcctcc  aaaatcggat
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721  aagcaattta  ggtatgaaag  ccagctacag  atggtacagg  tgaccggctc  ctgataaat
781  gagtacttct  acgttgattt  cagagaatat  gaatatgatc  tcaaattggga  gtttccaaga
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961  gaaaaagcag  acagctctga  aagagaggca  ctcatgtcag  aactcaagat  gatgaccag
1021  ctgggaagcc  acgagaatat  tgtgaacctg  ctggggcgct  gcacactgtc  aggaccaatt
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1201  actttccaat  cacatccaaa  ttccagcatg  cctggttcaa  gagaagttca  gatacacccg
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1381  cttctttgct  ttgcatatca  agttgccaaa  ggaatggaat  ttctggaatt  taagtctgtg
1441  gttcacagag  acctggccgc  caggaacgtg  cttgtcacc  acgggaaagt  ggtgaagata
1501  tgtgactttg  gattggctcg  atatatcatg  agtgattcca  actatgttgt  caggggcaat

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1681 ccttaccctg gcattccggt tgatgctaac ttctacaaac tgattcaaaa tggatttaaa
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1801 tttgactcaa ggaaacggcc atccttcctt aatttgactt cgttttttagg atgtcagctg
1861 gcagatgcag aagaagcgat gtatcagaat gtggatggcc gtgtttcggg atgtcctcac
1921 acctaccaa acaggcgacc tttcagcaga gagatggatt tggggctact ctctccgag
1981 gctcaggtcg aagattcgta g
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