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## Certificate of Analysis

### Flt1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Catalogue # 14-562

Lot # 1660666

From bulk lot # D7MN017U

**Product Description:** N-terminal 6His-tagged recombinant human Flt1 residues 783–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni<sup>2+</sup>/NTA agarose. Purity 44% by SDS-PAGE and Coomassie blue. MW = 66kDa.

**Specific Activity (lot# 1660666):** 70U/mg, where one unit of Flt1 activity is defined as 1nmol phosphate incorporated into 250µM IGFtide (KKKSPGEYVNIEFG) per minute at 30°C with a final ATP concentration of 100µM.

**Formulation:** 10µg of enzyme in 9.4µl of 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.

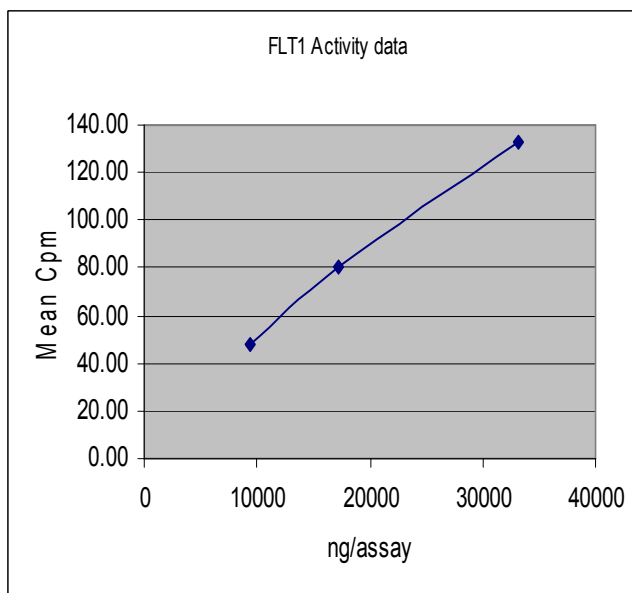
**Storage and Stability:** Store at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

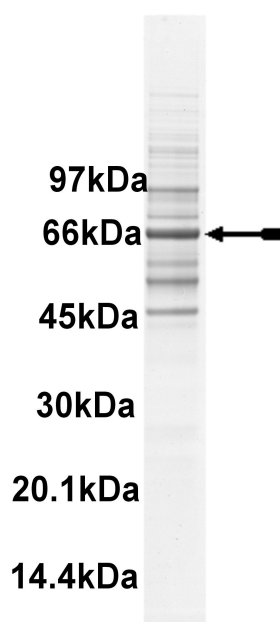
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 48–133ng of this lot of enzyme phosphorylated 250µM IGFtide 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 Flt1 with 44% amino acid coverage of 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 Flt1, active.

## Kinase Assay Protocol

### Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **IGFtide (KKKSPGEYVNIEFG):** Use at a final assay concentration of 250 $\mu$ M. Prepare a 2.5mM stock and add 2.5 $\mu$ l of stock per assay point.
3. **Flt1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 48–133ng per assay point.
4. **[ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<sup>33</sup>P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

### Assay Procedure (96 well plate format):

1. Add 5 $\mu$ l of 5 x reaction buffer to wells.
2. Add 2.5 $\mu$ l of **IGFtide**.
3. Add **2.5 $\mu$ l (48–133ng) Flt1, active**.
4. Add 5 $\mu$ l of dH<sub>2</sub>O.
5. Add 10 $\mu$ l of diluted [ $\gamma$ -<sup>33</sup>P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 $\mu$ l of 3% phosphoric acid.
8. Transfer a 10 $\mu$ 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 $\mu$ l of 30% phosphoric acid.

## Flt1Sequence Information

**Protein** human Flt1  
**Tags** N-terminal 6His  
**Native sequence** M29 of the recombinant protein is equivalent to M783 of human Flt1  
**Accession number** GenBank AF063657

### Recombinant Flt1 amino acid sequence:

```
1 MSYYHHHHHH DYDIPTTENL YFQGAMGSMK RSSSEIKTDY LSIIMDPDEV PLDEQCERLP
61 YDASKWEFAR ERLKLGKSLG RGAFGKVVQA SAFGIKKSPT CRTVAVKMLK EGATASEYKA
121 LMTELKILTH IGHHLNVVNL LGACTKQGGP LMVIVEYCKY GNLSNYLKSK RDLFFLNKDA
181 ALHMEPKKEK MEPGLEQGKK PRLDSVTSSE SFASSGFQED KSLSDVEEEE DSDGFYKEPI
241 TMEDLISYSF QVARGMEFLS SRKCIHRDLA ARNILLSENN VVKICDFGLA RDIYKNPDYV
301 RKG DTRLPLK WMAPESIFDK IYSTKSDVWS YGVLLWEIFS LGGSPYPGVQ MDEDFC SRLR
361 EGMRRAPEY STPEIQIML DCWHRDPKER PRFAELVEKL GDLLQANVQQ DGKDIYPINA
421 ILTGNSGFTY STPAFSEDFE KESISAPKFN SGSSDDVRYV NAFKFMSLER IKTFEELLPN
481 ATSMFDDYQG DSSTLLASPM LKRFTWTD SK PKASLKIDLR VTSKSKESGL SDVSRPSFCH
541 SSCGHVSEGK RRFTYDHAEL ERKIACCSPP PDYNSVVLYS TPPI
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### Recombinant Flt1 nucleotide sequence:

```
1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatggg atccatgaaa aggtcttctt ctgaaataaa gactgactac
121 ctatcaatta taatggaccc agatgaagtt cctttggatg agcagtgtga ggggctccct
181 tatgatgcca gcaagtggga gttgcccgg gagagactta aactgggcaa atcacttgga
241 agaggggctt ttggaaaagt ggttcaagca tcagcatttg gcattaagaa atcacctacg
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541 gcactacaca tggagcctaa gaaagaaaa atggagccag gcctggaaca aggcaagaaa
601 ccaagactag atagcgtcac cagcagcga agctttgcca gctccggctt tcaggaagat
661 aaaagtttga gtgatgttga ggaagaggag gattctgacg gtttctaaa ggagcccatc
721 actatggaag atctgatttc ttacagtttt caagtggcca gaggcattga gttcctgtct
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1381 aatgctttca agttcatgag cctggaaaaga atcaaacct ttgaagaact tttaccgaat
1441 gccacctcca tgtttgatga ctaccagggc gacagcagca ctctgttggc ctctcccatg
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1561 gtaaccagta aaagtaagga gtcggggctg tctgatgtca gcaggcccag tttctgccat  
1621 tccagctgtg ggcacgtcag cgaaggcaag cgcaggttca cctacgacca cgctgagctg  
1681 gaaaggaaaa tcgcgtgctg ctccccgcc ccagactaca actcgggtggt cctgtactcc  
1741 accccacca tctag

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