

## Certificate of Analysis

### Flt-1, active

(recombinant enzyme expressed in Sf21 insect cells)

Catalog # 14-562

Lot # D7MN032BU

**Product Description:** N-terminal 6His-tagged recombinant human Flt-1 residues 783–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni<sup>2+</sup>/NTA agarose. Purity 43% by SDS PAGE and Coomassie blue. MW = 66kDa.

**Specific Activity (lot D7MN032BU):** 94 U/mg, where one unit of Flt-1 activity is defined as 1 nmol phosphate incorporated into 250µM IGFtide (KKKSPGEYVNIEFG) per minute at 30°C with a final ATP concentration of 100µM.

**Formulation:** 10µg of enzyme in 100µl 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.

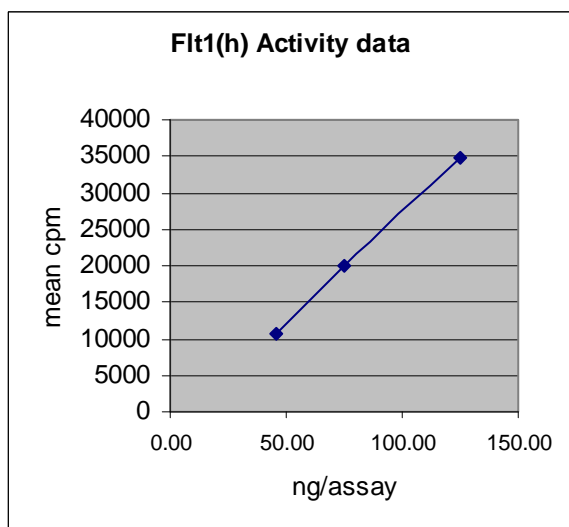
**Storage and Stability:** Store at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

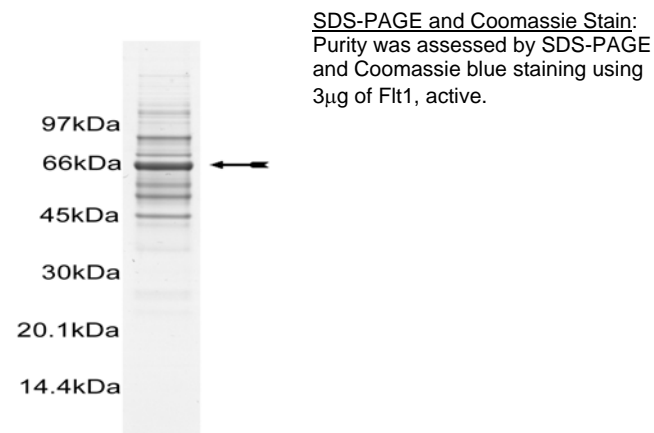
**FOR IN VITRO RESEARCH USE ONLY  
 NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 45 – 125 ng 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 identity as Flt1 with 44% amino acid coverage of the translated sequence listed on page 3.



### 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 to reaction mixture.
3. **Fit-1:** Dilute in 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 45 – 125 ng 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 - 800 cpm/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 (45 – 125 ng) Fit-1, 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 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 to CPM of control samples that contain all assay components plus 1 $\mu$ l of 30% phosphoric acid.

### Flt-1 Sequence Information

**Protein** human Flt-1  
**Tags** N-terminal 6His-tag  
**Native sequence** M29 of the recombinant protein is equivalent to M783 of human Flt1.  
**Accession number** GenBank AF063657

#### Recombinant Flt-1 amino acid sequence:

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1 MSYYHHHHHH DYDIP'TTENL 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 YGVLLEWEIFS LGGSPYPGVQ MDEDFCSRLR
361 EGMRMRAPEY STPEIYQIML DCWHRDPKER PRFAELVEKL GDLLQANVQQ DGKDYIPINA
421 ILTGNSGFTY STPAFSEDFE KESISAPKFN SGSSDDVRYV NAFKFMSLER IKTFEELLPN
481 ATSMFDDYQG DSSTLLASPM LKRFTWTD SK PKASLKIDLR VTSKSKESGL SDVSRPSFCH
541 SSCGHVSEGK RRF'TYDHAEL ERKIACCSPP PDYNSVVLYS TPPI
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#### Recombinant Flt-1 nucleotide sequence:

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1 atgtcgact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatggg atccatgaaa aggtcttctt ctgaaataaa gactgactac
121 ctatcaatta taatggacct agatgaagtt cctttggatg agcagtgatg gcggctccct
181 tatgatgcca gcaagtggga gtttgcccgg gagagactta aactgggcaa atcacttgga
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