

Certificate of Analysis

Flt-1, active

(recombinant enzyme expressed in Sf21 insect cells)

Catalog # 14-562

Lot # 33032U

Product Description: N-terminal 6His-tagged recombinant human Flt-1 residues 783–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 58% by SDS PAGE and Coomassie blue. MW = 66kDa.

Specific Activity (lot 33032U): 100U/mg, where one unit of Flt-1 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 5µl 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.

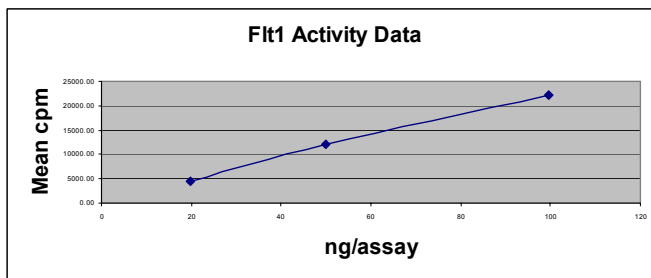
Storage and Stability: Store at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

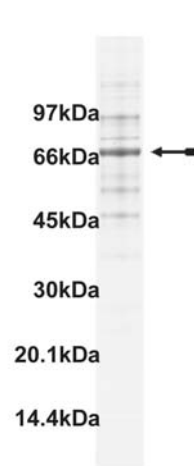
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 99.6 – 19.9ng of this lot of enzyme phosphorylated 250µM IGFtide in the assay described on page two. Assay background was 459 CPM, and was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as Flt1 with 51% amino acid coverage of the translated sequence listed on page 3.



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 μ M. Prepare a 2.5mM stock and add 2.5 μ l of stock to reaction mixture.
3. **Flt-1:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij 35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 99.6 – 19.9ng 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 - 800 cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer to wells.
2. Add 2.5 μ l of IGFtide.
3. Add **2.5 μ l (99.6 – 19.9ng) Flt-1, 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 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 to CPM of control samples that contain all assay components plus 1 μ 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 DYDIPTTENL YFQGAMGSMK RSSSEIKTDY LSIIMDPDEV PLDEQCERLP
61 YDASKWEFAR ERLKLGKSLG RGAFGKVVQA SAFGIKKSPT CRTVAVKMLK EGATASEYKA
121 LMTELKILTH IGHHLNVVNL LGACTKQGGP LMVIVEYCKY GNLSNYLKS K RDLFFLNKDA
181 ALHMEPKKEK MEPGLEQGGK PRLDSVTSSE SFASSGFQED KSLSDVEEEE DSDGFYKEPI
241 TMEDLISYSF QVARGMEFLS SRKCIHRDLA ARNILLSENN VVKICDFGLA RDIYKNPDYV
301 RKG DTRLPLK WMAPESIFDK IYSTKSDVWS YGVLLWEIFS 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 SSCGHVSE GK RRF TYDHAE L ERKIACCSPP PDYNSVVLYS TPPI
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Recombinant Flt-1 nucleotide sequence:

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1 atgtcg tact accatcac caaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatggg atccatgaaa aggtcttctt ctgaaataaa gactgactac
121 ctatcaatta taatggacct agatgaagtt cctttggatg agcagtgatg gcggctccct
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