

Certificate of Analysis

Flt4, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-681, 14-681-K, 14-681M

Parent Lot # 31064U

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 Flt4, amino acids 800–end, expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose. Purity 43.7% by SDS-PAGE and Coomassie blue staining. MW = 90.9kDa.

Specific Activity (Parent lot# 31064U): 133U/mg, where one unit of Flt4, active activity is defined as 1nmol phosphate incorporated into 500µM (GGEEEEYFELVKKKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.741mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

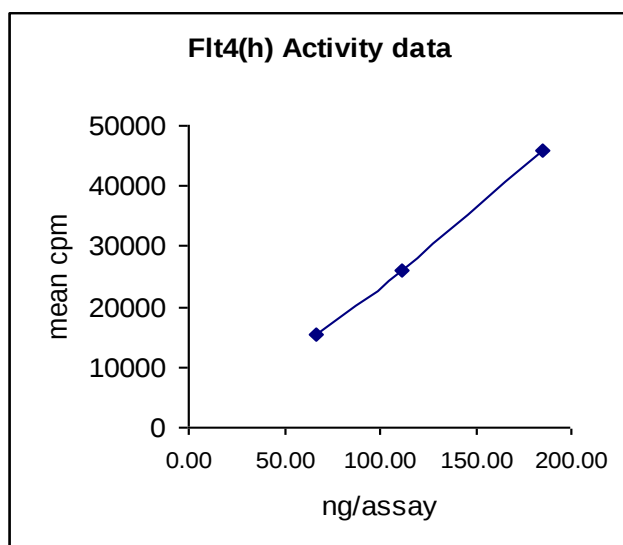
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

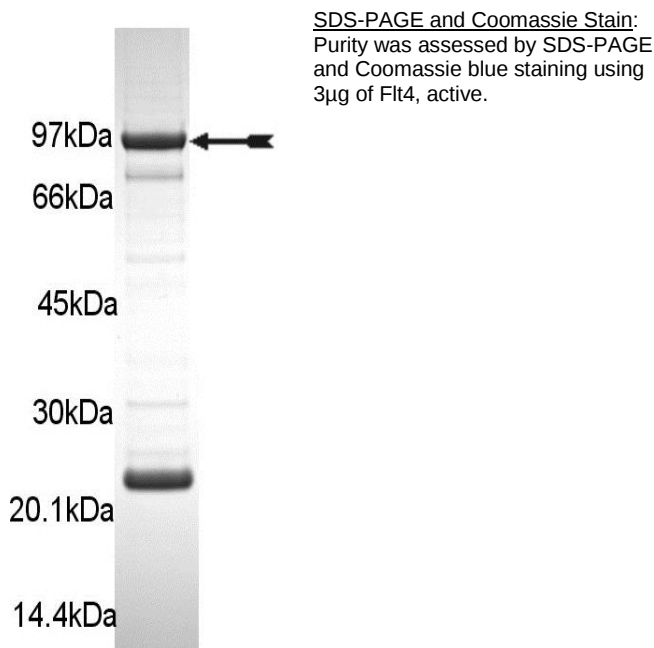
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 67–185ng of this lot of enzyme phosphorylated 500µM (GGEEEEYFELVKKKK) 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 Flt4 with the translated native sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **(GGEEEEYFELVKKKK):** Use at a final concentration of 500µM. Add 2.5µl of stock per assay point.
3. **Flt4, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 67–185ng 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 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of 500µM (GGEEEEYFELVKKKK).
3. Add **2.5µl (67–185ng) Flt4, active.**
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ-³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl 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 1µl of 30% phosphoric acid.

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Flt4 Sequence Information

<u>Protein</u>	Human Flt4
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M238 of recombinant protein is equivalent to M800 of human Flt4
<u>Accession number</u>	GenBank NM_182925. The recombinant protein contains the amino acid substitutions V1128L and H1146R with reference to NM_182925. These mutations are reported in GenBank U43143 and GenBank X68203.

Recombinant Flt4 amino acid sequence:

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1  MSPILGYWKI KGLVQPTRL L LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61  GDVKLTQSM A IIRYIADKH N MLGGCPKERA EISMLEGAVL DIRYGVSR I YSKDFETLKV
121 DFLSKLPEML KMFEDRLCH K TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAI PQID KYLKSSKYIA WPLQGWQATF GGDHPPKSD LEVLFQGPEF KGLRRQSMRR
241 PAHADIKTG Y LSIIMDPGEV PLEEQCEYLS YDASQWEFPR ERLHLGRVLG YGAFGKVVEA
301 SAFGIHKGS S CDTVAVKMLK EGATASEQRA LMSELKILIH IGNHLNVVNL LGACTKPQGP
361 LMVIVEFCK Y GNLSNFLRA K RDAFSPCAEK SPEQRGRFRA MVELARLDRR RPSGSSDRVLF
421 ARFSKTEGGA RRASPDQEA E DLWLSPLTME DLVCYSFQVA RGMEFLASRK CIHRDLAARN
481 ILLSESDVVK ICDFGLARDI YKDPDYVRKG SARLPLKWMA PESIFDKVYT TQSDVWSFGV
541 LLWEIFSLGA SPYPGVQINE EFCQRLRDGT RMRAPELATP AIRRIMLNCW SGDPKARPAF
601 SELVEILGDL LQGRGLQEEE EVCMA PRSSQ SSEEGSFSQV STMALHIAQA DAEDSPPSLQ
661 RHSLAARYYN WVSFPGCLAR GAETR GSSRM KTFEEFPMTT TTYKGSVDNQ TDSGMVLASE
721 EFEQIESRHR QESGF SCKGP GQNVAVTRAH PDSQGRRRRP ERGARGGQVF YNSEYGE LSE
781 PSEEDHCSPS ARVTFFTDNS Y

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Recombinant Flt4 nucleotide sequence:

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1  atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61  ttggaatata ttgaagaaaa atatgaagag catttgatat agcgcatga aggtgataaa
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1321 gacctgtggc tgagcccgt gacctggaa gatcttgtct gctacagctt ccaggtggcc
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1561 cctgaaagca tcttcgacaa ggtgtacacc acgcagagtg acgtgtggtc ctttggggtg
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2401 tactaa

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