

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.

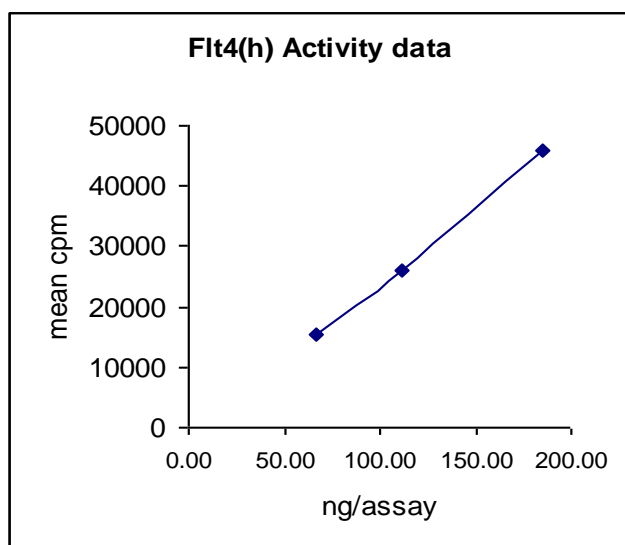
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

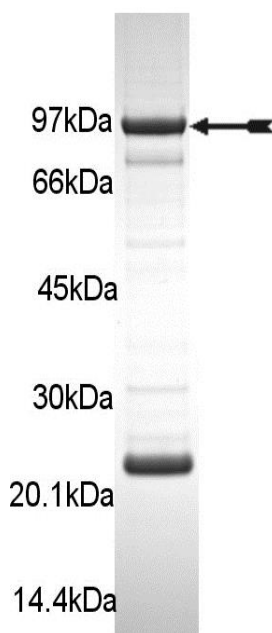
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

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.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of Flt4, active.

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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 500 μ M. Add 2.5 μ l 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 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of 500 μ M (GGEEEEYFELVKKKK).
3. Add 2.5 μ l (67–185ng) Flt4, 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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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  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAIQID  KYLKSSKYIA  WPLQGWQATF  GGDHPPKSD  LEVLFQGPFE  KGLRRQSMRR
241  PAHADIKTGY  LSIIMDPGEV  PLEEQCEYLS  YDASQWEFPR  ERLHLGRVLG  YGAFGKVVVEA
301  SAFGIHKGSS  CDTVAVKMLK  EGATASEQRA  LMSELKILIH  IGNHLNVVNL  LGACTKPQGP
361  LMVIVEFCKY  GNLSNFLRAK  RDAFSPCAEK  SPEQRGRFRA  MVELARLDRR  RPGSSDRVLF
421  ARFSKTEGGA  RRASPDQEA  DLWLSPLTME  DLVCYSFQVA  RGMEFLASRK  CIHRDLAARN
481  ILLSESDVVK  ICDFGLARDI  YKDPDYVRKG  SARLPLKWMA  PESIFDKVYT  TQSDVVSFGV
541  LLWEIFSLGA  SPYPGVQINE  EFCQRLRDGT  RMRAPELATP  AIRRIMLNCW  SGDPKARPAF
601  SELVEILGDL  LQGRGLQEEE  EVCMAPRSSQ  SSEEGSFSQV  STMALHIAQA  DAEDSPPSLQ
661  RHSLAARYYN  WVSFPGCLAR  GAETRGSARM  KTFEEFPMT  TTYKGSVDNQ  TDSGMVLASE
721  EFEQIESRHR  QESGFSCCKG  QONVAVTRAH  PDSQGRRRRP  ERGARGGQVF  YNSEYGE LSE
781  PSEEDHCSPS  ARVTFFTDNS  Y

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Recombinant Flt4 nucleotide sequence:

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2401 tactaa
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