

Certificate of Analysis

Fms, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-551, 14-551-K, 14-551M

Parent Lot # 1625861

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 6His-tagged, recombinant, human FMS, amino acids 538–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 94.1% by SDS-PAGE and Coomassie blue staining. MW = 50.2kDa.

Specific Activity (Parent lot# 1625861): this lot showed a minimum activity of 317U/mg, where one unit of FMS, active activity is defined as 1nmol phosphate incorporated into 250μM (KKKSPGEYVNIEFG) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 1.282mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

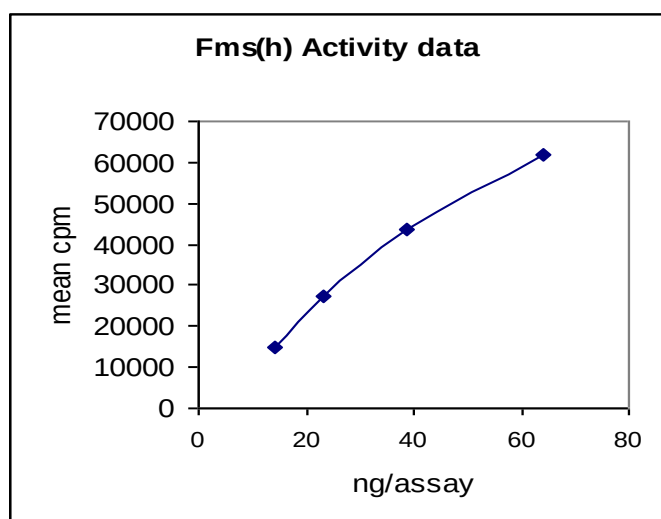
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

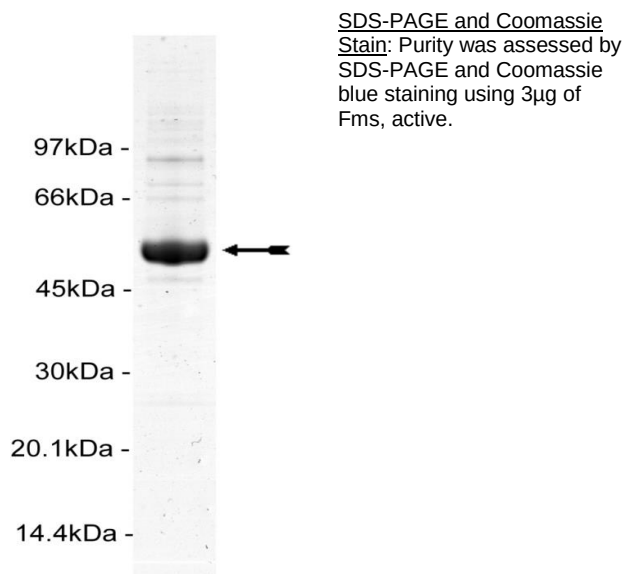
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 14–64ng of this lot of enzyme phosphorylated 250μM (KKKSPGEYVNIEFG) 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 FMS with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS pH 7.0, 1mM EDTA.
2. **(KKKSPGEYVNIEFG):** Use at a final assay concentration of 250 μ M. Make a 2.5mM stock. Use 2.5 μ l per assay point.
3. **FMS, active:** Dilute with 20mM MOPS pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 14–64ng 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 **(KKKSPGEYVNIEFG)**.
3. Add **2.5 μ l (14–64ng) FMS, active**.
4. Add 5 μ l 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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FMS Sequence Information

<u>Protein</u>	Human FMS
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	Y10 of the recombinant protein is equivalent to Y538 of human FMS
<u>Accession number</u>	GenBank U63963

Recombinant FMS amino acid sequence:

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1  MHHHHHHEFY  KYKQKPKYQV  RWKIIESYEG  NSYTFIDPTQ  LPYNEKWEFP  RNNLQFGKTL
61  GAGAFGKVVE  ATAFGLGKED  AVLKVAVKML  KSTAHADEKE  ALMSELKIMS  HLQHENIVN
121  LLGACTHGGP  VLVITEYCCY  GDLLNFLRRK  AEAMLGPSLS  PGQDPEGGVD  YKNIHLEKKY
181  VRRDSGFSSQ  GVDTYVEMRP  VSTSSNDSFS  EQDLDKEDGR  PLELRDLLHF  SSQVAQGMAF
241  LASKNCIHRD  VAARNVLLTN  GHVAKIGDFG  LARDIMNDSN  YIVKGNARLP  VKWMAPEISF
301  DCVYTVQSDV  WSYGILLWEI  FSLGLNPYPG  ILVNSKFYKL  VKDGYQMAQP  AFAPKNIYSI
361  MQACWALEPT  HRPTFQQICS  FLQEQAQEDR  RERDYNLPS  SSRSGSGSS  SSELEEESSS
421  EHLTCCEQGD  IAQPLLQPN  YQFC

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Recombinant FMS nucleotide sequence:

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1  atgcatcatc  accatcacca  tgaattctac  aagtataagc  agaagcccaa  gtaccaggtc
61  cgctggaaga  tcatcgagag  ctatgagggc  aacagttata  ctttcatcga  cccacgcag
121  ctgccttaca  acgagaagtg  ggagttcccc  cggaacaacc  tgcagtttgg  taagaccctc
181  ggagctggag  cctttgggaa  ggtggtggag  gccacggcct  ttggtctggg  caaggaggat
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1261  gagcacctga  cctgctgcga  gcaaggggat  atcgcccagc  ccttgctgca  gcccacaac
1321  tatcagttct  gctga

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