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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 Ni^{2+}/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 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.

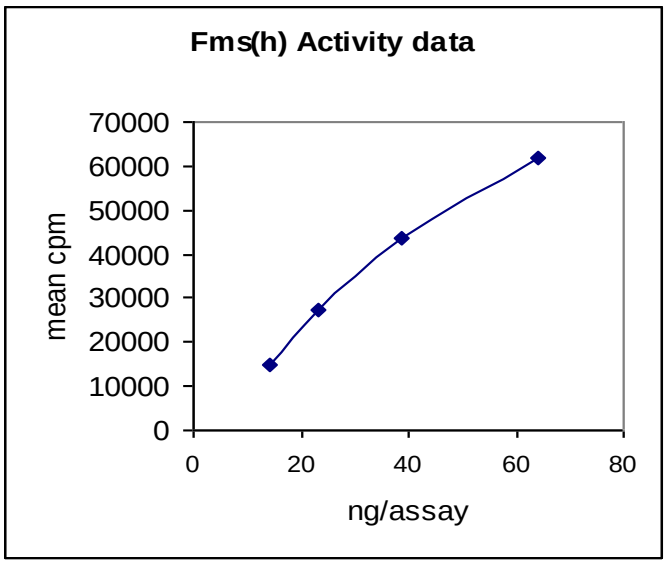
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

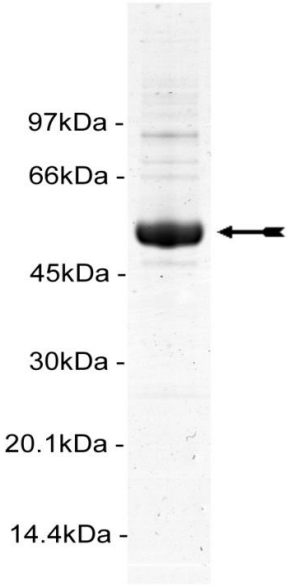
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 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.



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

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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 250µM. Make a 2.5mM stock. Use 2.5µl 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-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]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 **(KKKSPGEYVNIEFG)**.
3. Add **2.5µl (14–64ng) FMS, active**.
4. Add 5µl dH_20.
5. Add 10µl of diluted [γ-^{33}P] 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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FMS Sequence Information

Protein Human FMS

Tags *N*-terminal 6His

Native sequence Y10 of the recombinant protein is equivalent to Y538 of human FMS

Accession number GenBank U63963

Recombinant FMS amino acid sequence:

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  1 MHHHHHHEFY KYKQKPKYQV RWKIIESYEG NSYTFIDPTQ LPYNEKWEFP RNNLQFGKTL
 61 GAGAFGKVVE ATAFGLGKED AVLKVAVKML KSTAHADEKE ALMSELKIMS HLGQHENIVN
121 LLGACTHGGP VLVITEYCCY GDLLNFLRRK AEAMLGPSLS PGQDPEGGVD YKNIHLEKKY
181 VRRDSGFSSQ GVDTYVEMRP VSTSSNDSFS EQDLDKEDGR PLELRDLLHF SSQVAQGMAF
241 LASKNCIHRD VAARNVLLTN GHVAKIGDFG LARDIMNDSN YIVKGNARLP VKWMAPESIF
301 DCVYTVQSDV WSYGILLWEI FSLGLNPYPG ILVNSKFYKL VKDGYQMAQP AFAPKNIYSI
361 MQACWALEPT HRPTFQQICS FLQEQAQEDR RERDYTNLPS SSRSGGSGSS SSELEEESSS
421 EHLTCCEQGD IAQPLLQPNN 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 ccccacgcag
 121 ctgccttaca acgagaagtg ggagttcccc cggaacaacc tgcagtttgg taagaccctc
 181 ggagctggag cctttgggaa ggtggtggag gccacggcct ttggtctggg caaggaggat
 241 gctgtcctga aggtggctgt gaagatgctg aagtccacgg cccatgctga tgagaaggag
 301 gccctcatgt ccgagctgaa gatcatgagc cacctgggcc agcacgagaa catcgtcaac
 361 cttctgggag cctgtaccca tggaggccct gtactggtca tcacggagta ctgttgctat
 421 ggcgacctgc tcaactttct gcgaaggaag gctgaggcca tgctgggacc cagcctgagc
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 541 gtccgcaggg acagtggctt ctccagccag ggtgtggaca cctatgtgga gatgaggcct
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1261 gagcacctga cctgctgcga gcaaggggat atcgcccagc ccttgctgca gcccaacaac
1321 tatcagttct gctga
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Reviewed and approved by site quality representative.