

Certificate of Analysis

Fms (Y969C), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-820, 14-820-K, 14-820M

Parent Lot # D8CN041N

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, containing the mutation Y969C, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose.

In vitro studies have demonstrated the transforming potential of the Fms point mutation Y969C. This mutation has been reported in a number of patients diagnosed with acute myeloblastic leukaemia (AML). (Ridge *et al.*, (1990), PNAS, **87**, 1377-1380 and Baker *et al.*, (1994), Leukaemia, **8**, 141-150). Purity 95% by SDS-PAGE and Coomassie blue staining. MW = 50.2kDa.

Specific Activity (Parent lot# D8CN041N): 255U/mg, where one unit of Fms (Y969C), active activity is defined as 1nmol phosphate incorporated into 100μM (EEEEEEEEEEEEYYIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIKKK) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 2.828mg/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.

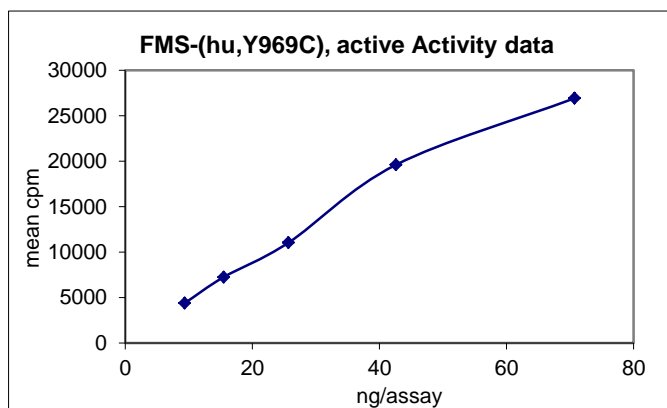
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

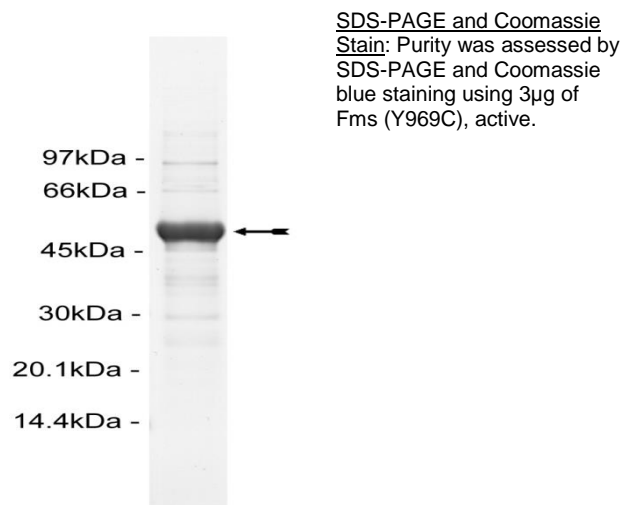
FOR IN VITRO RESEARCH USE ONLY NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 9.3–70.7ng of this lot of enzyme phosphorylated 100μM (EEEEEEEEEEEEYYIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIKKK) 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 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/NaOH pH7.0, 1mM EDTA.
2. **(EEEEEEEEEEYYIIEEEEEEYEEEEYYEE EEEEKKKK):** Use at a final assay concentration of 100 μ M. Prepare a 1mM stock and add 2.5 μ l of stock per assay point.
3. **Fms (Y969C), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 9.3–70.7ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x MgAc/[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 **(EEEEEEEEEEYYIIEEEEEEYEEEEYYEE EEEEKKKK)**.
3. Add **2.5 μ l (9.3–70.7ng) Fms (Y969C), 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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Fms (Y969C) Sequence Information

<u>Protein</u>	human Fms (Y969C)
<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 (Y969C) 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 SSQVAQGMFAF
241 LASKNCIHRD VAARNVLLTN GHVAKIGDFG LARDIMNDSN YIVKGNARLP VKWMAPE SIF
301 DCVYTVQSDV WSYGILLWEI FSLGLNPYPG ILVNSKFYKL VKDGYQMAQP AFAPKNIYSI
361 MQACWALEPT HRPTFQQICS FLQEQAQEDR RERDYTNLPS SSRSGGSGSS SSELEEESSS
421 EHLTCCEQGD IAQPLLQPNN CQFC
  
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Recombinant Fms (Y969C) nucleotide sequence:

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1 atgcatcatc accatcacca tgaattctac aagtataagc agaagcccaa gtaccaggtc
61 cgctggaaga tcatcgagag ctatgagggc aacagttata ctttcatcga ccccacgcag
121 ctgccttaca acgagaagtg ggagttcccc cggacaacc 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 gtactggcca tcacggagta ctggtgctat
421 ggcgacctgc tcaactttct gcgaaggaag gctgaggcca tgctgggacc cagcctgagc
481 cccggccagg accccgaggg aggcgtcgac tataagaaca tccacctcga gaagaaatat
541 gtccgcaggg acagtggctt ctccagccag ggtgtggaca cctatgtgga gatgaggcct
601 gtctccactt cttcaaatga ctcttctct gagcaagacc tggacaagga ggatggacgg
661 cccctggagc tccgggacct gcttcaactt tccagccaag tagcccaggg catggccttc
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841 tacattgtca agggcaatgc ccgctgcct gtgaagtgga tggccccaga gagcatcttt
901 gactgtgtct acacggttca gacgacgctc tggctctatg gcatcctcct ctgggagatc
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1141 ttccttcagg agcaggccca agaggacagg agagagcggg actataccaa tctgccgagc
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1261 gacacactga cctgctgca gcaaggggat atcgcccagc cttgtctgca gccaacaac
1321 tgtcagttct gctga
  
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