

Status: Currently Official on 14-Feb-2025
Official Date: Official as of 01-Jan-2021
Document Type: USP Monographs
DocId: GUID-473D9F75-B39B-4B07-82CD-791261EBC13B_5_en-US
DOI: https://doi.org/10.31003/USPNF_M33170_05_01
DOI Ref: lop9c

© 2025 USPC
Do not distribute

Filgrastim

MTPLGPASSL PQSFLKLCLE QVRKIQGDGA ALQEKLCATY KLCHPEELVL
LGHSLGIPWA PLSSCPSQAL QLAGCLSQLH SGLFLYQGLL QALEGISPEL
GPTLDTLQLD VADFATTIWQ QMEELGMAPA LQPTQGAMPA FASAFQRRAG
GVLVASHLQS FLEVSRYVLR HLAQP

C₈₄₅H₁₃₃₉N₂₂₃O₂₄₃S₉ 18,799 daltons CAS RN®: 121181-53-1; UNII: PVI5M0M1GW.

DEFINITION

Filgrastim is a recombinant form of human granulocyte colony-stimulating factor (r-metHuG-CSF). It is a single chain, 175 amino acid nonglycosylated polypeptide produced by *Escherichia coli* bacteria transfected with a gene encoding a methionyl human granulocyte colony-stimulating factor. When prepared as a drug substance, it contains NLT 0.9 mg/mL of Filgrastim. Formulation contains one or more suitable buffering and/or stabilizing agents. The presence of host cell DNA and protein in Filgrastim is process-specific. The capability of the process to clear host-derived DNA and protein requires validation and is determined by validated methods. It has a biological potency of NLT 80% and NMT 125% relative to standard on a mass-to-mass basis.

IDENTIFICATION

- **A.** It meets the requirements in the Assay.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Organic Impurities, Related Compounds*.

Change to read:

- **C. PEPTIDE MAPPING**

(See [Biotechnology-Derived Articles—Peptide Mapping \(1055\)](#).)

Solution A: [Water](#) and [trifluoroacetic acid](#) (1000:1)

Solution B: Transfer 100 mL of [water](#) to a 1000-mL volumetric flask, add 1 mL of [trifluoroacetic acid](#), and dilute with [acetonitrile](#) to volume.

Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	98	2
2	98	2
30	70	30
75	40	60
90	2	98
100	2	98
101	98	2
120	98	2

Tris buffer: Prepare a 0.5 M [tris\(hydroxymethyl\) aminomethane](#) solution, and adjust with [hydrochloric acid](#) to a pH of 8.1.

Methylamine solution: Dissolve 0.27 g of [methylamine hydrochloride](#) in 10 mL of [water](#).

DTT solution: 30.9 mg/mL of [dithiothreitol](#) in [water](#), freshly prepared

Digestion solution: Dissolve 0.30 g of [urea](#) in 200 µL of *Tris buffer*, 100 µL of *Methylamine solution*, 50 µL of *DTT solution*, and 420 µL of [water](#). Overlay with nitrogen, and use immediately.

Endoproteinase Glu-C solution: 0.2 µg/µL of [endoproteinase Glu-C](#) in [water](#). Use immediately.

TFA solution: [Water](#) and [trifluoroacetic acid](#) (100:5)

Standard solution: Prepare a solution containing 80 µg of ▲[USP Filgrastim System Suitability RS](#)▲ (IRA 1-Jan-2021) and 200 µL of *Digestion solution* in a suitable tube. Add [water](#) to a final volume of 390 µL. Add 10 µL of *Endoproteinase Glu-C solution*. Cap the tube, mix well, and incubate at approximately 25° for 18 h. Add 18 µL of *TFA solution*.

Sample solution: ▲Prepare a solution containing 80 µg of filgrastim and 200 µL of *Digestion solution* in a suitable tube. Add [water](#) to a final volume of 390 µL. Add 10 µL of *Endoproteinase Glu-C solution*. Cap the tube, mix well, and incubate at approximately 25° for 18 h. Add 18 µL of *TFA solution*.▲ (IRA 1-Jan-2021)

Chromatographic system

(See [Chromatography \(621\), System Suitability](#).)

Mode: LC

Detector: UV 214 nm

Column: 2.1-mm × 25-cm; packing [L26](#)

Column temperature: 40°

Flow rate: 0.2 mL/min

Injection volume: 70 µL

System suitability requirements: Eight major peaks should be present in each chromatogram as illustrated in the reference chromatogram provided with ▲[USP Filgrastim System Suitability RS](#)▲ (IRA 1-Jan-2021). The absolute difference in retention time of each of the eight major peaks between the two *Standard solution* chromatograms must be ≤0.5 min. The difference in retention time of each of the eight major peaks between the *Sample solution* chromatogram and the average of the *Standard solution* chromatograms must be ≤0.5 min. The relative difference in peak height of each of the eight major peaks between the two *Standard solution* chromatograms must be ≤15%.

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Condition the *Chromatographic system* by running at least two blank gradient programs before injecting the digests. Separately inject the *Standard solution*, *Sample solution*, and *Standard solution* again, and record the responses of each peak.]

Acceptance criteria: The chromatographic profile of the *Sample solution* is similar to that of the *Standard solution*. The relative difference in peak height between the normalized sample peak height (normalized by total peak height versus the average total peak height of the *Standard solution* chromatograms) and the average standard peak height of each of the eight major peaks must be ≤15%.

ASSAY

Change to read:

• POTENCY

Medium A¹: [RPMI 1640](#) modified to contain 2 mM [L-glutamine](#), 2 g/L of [sodium bicarbonate](#), 4.5 g/L of [glucose](#), 10 mM ▲[N-\(2-Hydroxyethyl\)piperazine-N'-\(ethanesulfonic acid\)](#)▲ (IRA 1-Jan-2021) [HEPES], 1 mM [sodium pyruvate](#), 0.05 mM [2-mercaptoethanol](#), and 5% heat-inactivated fetal bovine serum (FBS). The [2-mercaptoethanol](#) is prepared fresh and added immediately before use.

Medium B: [RPMI 1640](#) modified to contain 2 mM [L-glutamine](#), 2 g/L of [sodium bicarbonate](#), 4.5 g/L of [glucose](#), 10 mM HEPES, 1 mM [sodium pyruvate](#), 0.05 mM [2-mercaptoethanol](#), and 1% heat-inactivated FBS. The [2-mercaptoethanol](#) is prepared fresh and added immediately before use.

Medium C: Prepare a mixture of *Medium A* with 20 ng/mL of [interleukin 3](#) (IL-3).

Medium D: Prepare a mixture of *Medium A* with 1 ng/mL of [granulocyte colony-stimulating factor](#) (G-CSF).

Substrate solution: Reconstitute the luminescence substrate² with the supplied buffer. Aliquot the solution and store below -65° for up to 2 months.

Standard solution: 0.5 ng/mL of ▲[USP Filgrastim for Bioassay RS](#)▲ (IRA 1-Jan-2021) in *Medium B*. [NOTE—Do not use single step dilutions of more than 1:100 and smaller transfer volumes than 40 µL while performing serial dilutions. Mix gently and thoroughly (do not vortex).]

Positive control solution: 10 ng/mL of ▲[USP Filgrastim for Bioassay RS](#)▲ (IRA 1-Jan-2021) in *Medium B*

Sample solution: 0.5 ng/mL of Filgrastim in *Medium B*. [NOTE—Do not use single step dilutions of more than 1:100 and smaller transfer volumes than 40 µL while performing serial dilutions. Mix gently and thoroughly (do not vortex).]

Cell culture preparation: Adapt [M-NFS-60 cells](#)³ to growth in [G-CSF](#). Culture the cells at 5% carbon dioxide (CO₂) and 37°. Cells should be passaged two times per week and reseeded at a density of 3 × 10⁴ cells/mL for 3 days or 1 × 10⁴ cells/mL for 4 days. Passage the cultures in *Medium C* until passage two. Transfer the cells into *Medium D*, and passage the cultures until passage 11. After passage 11, the cells are defined as [G-CSF](#) adapted and can be used in the Assay or can be banked. Bank the cells at a density of approximately 1.5 × 10⁶ cells/mL in banking medium [90% FBS, 10% [dimethyl sulfoxide](#) (DMSO)]. Upon thawing the banked cells, culture the cells for 2–3 passages in *Medium D* before using in the Assay.

Cell suspension: Wash the cells twice with [phosphate buffered saline](#) (PBS), and adjust the cell concentration to 1 × 10⁵ cells/mL in *Medium B*.

Preparation of cells for analysis: Use a 96-well flat-bottomed black microtiter plate (optically clear well bottoms) with wells arranged in eight rows (labeled A through H) with 12 wells (numbered 1 to 12) in each row. Place 50 µL of *Cell suspension* into all wells of the 96-well microtiter plate except the wells of column 1 (A1–H1, blank). Column 1 is filled with 50 µL of *Medium B*. Incubate the plate at 37° in the incubator until the *Standard solution* and the *Sample solution* are ready, for a maximum of 1 h.

Preparation of diluted Standard solution and Sample solution: Use a 96-well round-bottom microtiter plate with wells arranged in eight rows (labeled A through H) with 12 wells (numbered 1 to 12) in each row. Dispense 100 µL of *Medium B* into each well of the plate except column 12 (wells A12–H12, positive control) and A2–A10. Pipet 200 µL of *Standard solution* into wells A3, A6, and A9; and 200 µL of *Sample solution* into wells A2, A5, and A8. Two samples can be prepared on one plate. The second sample can be added to wells A4, A7, and A10. Pipet 100 µL of *Positive control solution* into all wells of the last column (A12–H12). Using a multi-channel pipet (12 channels), perform two-fold dilutions on the plate. Aspirate 100 µL from the first row (A2–A10), transfer to the second row, and mix three times. Then aspirate 100 µL from the second row, transfer to the third row, and mix three times. Repeat this procedure across the entire plate until row H. Discard the 100 µL aspirated from the last row.

System suitability requirements: The signal-to-noise ratio between the mean chemiluminescence signal of the positive control and the mean chemiluminescence signal of the negative control (A11–H11) should be ≥3. The number of technical outliers may not exceed four per curve.

Analysis: Using a multi-channel pipet, transfer 50 µL from each well of the round-bottom microtiter plate (containing the *Standard solution* and *Sample solution* serial dilutions, blanks, and positive and negative controls) into the same well of the black microtiter plate containing the cell suspension starting with the lowest concentration. Mix three times. Incubate the black microtiter plate in a humidified CO₂ incubator for 31 ± 2 h at 37° with 5% CO₂. Add 100 µL of reconstituted *Substrate solution* to all wells. Incubate the plate for 15 min at room temperature while gently agitating the plate on a plate shaker. Incubate the plate for an additional 15 min at room temperature without shaking. Read the plate in a microtiter plate luminescence reader.

Calculations: For each sample, first calculate the relative potency of Filgrastim (in percent) using statistical methods for parallel-line assays, then calculate potency in IU/mL. The statistical tests for linearity, slope, and parallelism for each sample compared to the standard have to be passed at the 95% level. The confidence limit must be within 75% and 133% of the estimated potency.

Acceptance criteria: The mean estimated potency is NLT 80% and NMT 125% of the stated potency.

IMPURITIES

Change to read:

• ORGANIC IMPURITIES, RELATED COMPOUNDS

Solution A: [Water](#) and [trifluoroacetic acid](#) (1000:1)

Solution B: Transfer 100 mL of [water](#) to a 1000-mL volumetric flask, add 1 mL of [trifluoroacetic acid](#), and dilute with [acetonitrile](#) to volume.

Mobile phase: See [Table 2](#).

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	60	40
30	20	80
35	20	80
45	60	40
55	60	40

Standard solution: 0.75 mg/mL of ▲[USP Filgrastim System Suitability RS](#)▲ (IRA 1-Jan-2021) in [water](#)

Sample solution: 0.75 mg/mL of Filgrastim in [water](#)

Chromatographic system

(See [Chromatography \(621\), System Suitability](#).)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; packing [L26](#)

Column temperature: 60°

Flow rate: 0.8 mL/min

Injection volume: 33 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative retention times: About 0.91 for oxidized Filgrastim 1, about 0.98 for oxidized Filgrastim 2, and about 1.04 for reduced Filgrastim relative to the major peak

Relative standard deviation: The relative standard deviation of the total area (main peak and minor product related peaks, excluding the void peak) and the relative standard deviation of the retention time of the major Filgrastim peak for replicate injections is NMT 5%.

Analysis