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Sargramostim

APARSPSPST QPWEHVNAIQ EALRLLNLSR DTAEMNETV EWISEMFDLQ
EPTCLQTRLE LYKQGLRGSL TKLKGPLTMM ASHYKQHCPP TPETSCATQI
ITFESFKENL KDFLLVIPFD CWEPVQE

C₆₃₉H₁₀₀₂N₁₆₈O₁₉₆S₈ (protein moiety) 14,414 CAS RN®: 123774-72-1; UNII: 5TAA004E22.

DEFINITION
Sargramostim is a single-chain, glycosylated polypeptide of 127 amino acid residues expressed from *Saccharomyces cerevisiae*. The glycoprotein primarily consists of three molecular species having relative molecular weights of approximately 19,500; 16,800; and 15,500 due to different levels of glycosylation. Sargramostim has the property of generating granulocyte, macrophage, and mixed granulocyte macrophage colonies from hematopoietic progenitor cells found in bone marrow. It possesses the primary sequence of the natural form of granulocyte-macrophage colony-stimulating factor with a substitution in the amino acid residue at position 23 (Leu₂₃ in place of Arg₂₃). It has a biological potency of NLT 73.0% and NMT 146.0% of the potency stated on the label, the potency being 5.6 million USP Sargramostim Units/mg of protein. The presence of host cell DNA and host cell protein impurities in Sargramostim is process-specific; the limits of these impurities are determined by validated methods.

- IDENTIFICATION**
- A.** The retention times of the peaks of the *Sample solution* do not differ by more than 0.5 min from those of the *Standard solution*, as obtained in the test for *Chromatographic Purity*.
 - B. PEPTIDE MAPPING**
Solution A: Trifluoroacetic acid and water (1:1000), filtered and degassed
Solution B: Trifluoroacetic acid and acetonitrile (1:1000), filtered and degassed
Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
35	65	35
50	35	65

Digestion solution: Dissolve 29.4 mg of calcium chloride and 1.8 mg of β-alanine in 2 mL of water. Adjust with hydrochloric acid to a pH of 4.0. Add 0.4 mg of trypsin, and mix.

Standard solution: 500 µg/mL of [USP Sargramostim RS](#) in water. Transfer 100 µL of this solution to a clean test tube, and add 11 µL of pH 7.6 buffer solution (see [Reagents, Indicators, and Solutions—Buffer Solutions](#)) containing 0.1 M tris(hydroxymethyl)aminomethane. Add 25 µL of *Digestion solution*, and incubate at 37° for 2 h. Quench the reaction by adding 3 µL of 20% trifluoroacetic acid.

Sample solution: 500 µg/mL of Sargramostim in water. Transfer 100 µL of this solution to a clean test tube, and add 11 µL of pH 7.6 buffer solution (see [Reagents, Indicators, and Solutions—Buffer Solutions](#)) containing 0.1 M tris(hydroxymethyl)aminomethane. Add 25 µL of *Digestion solution*, and incubate at 37° for 2 h. Quench the reaction by adding 3 µL of 20% trifluoroacetic acid.

Chromatographic system
(See [Chromatography \(621\), System Suitability](#).)
Mode: LC

Detector: UV 220 nm**Column:** 4.6-mm × 25-cm; 10-µm packing L1**Column temperature:** Ambient**Flow rate:** 1 mL/min**Injection volume:** 100 µL**Analysis****Samples:** *Standard solution* and *Sample solution*

Equilibrate the system with a *Mobile phase* consisting of 100% *Solution A*. Measure the responses for the eight major peaks as defined in the [USP Sargramostim RS](#) Data Sheet.

Acceptance criteria: The retention times of the peak responses of the *Sample solution* correspond to those of the *Standard solution* if the retention times of the corresponding peaks do not differ by more than 0.3 min; the peak area ratios for peaks 4, 8, and 10 are between 0.7 and 1.3; and no additional significant peaks or shoulders are found.

ASSAY**• PROCEDURE**

Iscove's Modified Dulbecco's medium: Prepare a mixture of the ingredients in the quantities shown in [Table 2](#) in sufficient water to obtain 1 L of medium, and sterilize by filtration.

Table 2

Calcium chloride	165.00 mg
Potassium chloride	330.00 mg
Potassium nitrate	0.076 mg
Magnesium sulfate	97.67 mg
Sodium chloride	4505.00 mg
Sodium bicarbonate	3024.00 mg
Monobasic sodium phosphate	125.00 mg
Sodium selenite	0.0173 mg
Glucose	4500.00 mg
HEPES (4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid)	5958.00 mg
Phenol red	15.00 mg
Sodium pyruvate	110.00 mg
L-Alanine	25.00 mg
L-Arginine hydrochloride	84.00 mg
L-Asparagine	28.40 mg
L-Aspartic acid	30.00 mg
L-Cystine dihydrochloride	91.24 mg
L-Glutamic acid	75.00 mg
L-Glutamine	584.00 mg