DATA SHEET

Recombinant Human Granulocyte Macrophage Colony Stimulating Factor GM CSF

(mGMP-rHuGM-CSF)

µg300 /Euro 385 :Price

Certificate of Analysis and Data Sheet

Description: mGMP Recombinant Human GM-CSF produced in E.Coli is a single, non-glycosylated,polypeptide chain containing 127 amino acids and having a molecular mass of 14477 Dalton. rHuGM-CSF is purified by proprietary chromatographic techniques.

Source: Escherichia Coli.

Physical Appearance: Sterile Filtered White lyophilized (freeze-dried) powder.

Formulation & Packaging: The protein was lyophilized after extensive dialysis against 2mM sodium phosphate buffer pH= 7.4±0.1.

Solubility: The lyophilized rHuGM-CSF is very soluble in water and most aqueous buffers below and above the isoelectric point (pI=4.95).

Stability: Lyophilized mGMPrHuGM-CSF although stable at room temperature, should be stored desiccated below 0°C. Reconstituted rHuGM-CSF is best stored refrigerated at 4°C.

Purity: **Greater than 99.0%** as determined by:

(a) Analysis by RP-HPLC (See Figure1).

(b) Anion-exchange FPLC.

(c) Analysis by reducing and non-reducing SDS-PAGE Silver Stained (See Figure 2).

(Limit of acceptance:≥98.0%. No more than 2% total impurities; no single impurity greater than 1%)

Amino Acid Composition: In total agreement with the expected amino acid composition of native human GM-CSF.

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Amino acid sequence: The sequence of the first five N-terminal amino acids was determined and was found to be Ala-Pro-Ala-Arg-Ser, conforming the sequence of native human GM-CSF. N-terminal methionine has been completely removed enzymatically.

Dimers and aggregates: Less than 1% as determined by silver stained SDS-PAGE gel analysis.

Biological Activity: mGMPrHuGM-CSF is fully biologically active when compared to standard.The ED_{50} as determined by the dose-dependant stimulation of the proliferation of human TF-1 cells (human erythroleukemic indicator cell line) is 0.1 ng/ml, corresponding to a Specific Activity of $9x10^6$IU/mg or 2 800 000 IU/ vial of 300 ug

Endotoxin: Less than 0.1 ng/µg (IEU/µg) of mGMPrHuGM-CSF.

Protein content: Protein quantitation was carried out by two independent methods:

1. UV spectroscopy at 280 nm using the absorbency value of 0.963 as the extinction coefficient for a 0.1% (1mg/ml) solution. This value is calculated by the PC GENE computer analysis program of protein sequences (IntelliGenetics).
2. Analysis by RP-HPLC, using a standard solution of GM-CSF as a Reference Standard.

Usage: This material is offered by Gentaur for research, laboratory or further manufacturing purposes adn DC culture

Cell lines

Hematopoietic cell lines K562, human erythroleukemia cell line (HEL), HL60, TF-1, Jurkat, Daudi, CEM, and ML1 (ATCC, Manassas, VA, USA) were cultured in the presence of 10% fetal bovine serum (FBS) (Euroclone, Milano, Italy) in Iscove's modified Dulbecco's medium (IMDM) or RPMI (Euroclone, Milano Italy) and 5% CO_2 in a humidified atmosphere of 5% O_2 in air at 37°C. TF-1 required the addition of 10 ng/ml of human granulocyte–macrophage colony-stimulating factor (GM-CSF, Leukomax; Schering-Plough, Basel, Switzerland).