

# **Recombinant human GM-CSF**

## **(Granulocyte-macrophage colony-stimulating factor)**

**Human recombinant protein expressed in *Nicotiana benthamiana***

**RF0036**

**Mol. Formula:** C699H1077N201O206S8

**Extinction coeff:** Abs 0.1% (1g/l) = 0.898

**Mol. Weight:** rHuman GM-CSF is a glycosylated polypeptide chain containing 127 amino acids (18-144 aa CSF2\_HUMAN P04141 ), fused to a 10His tag at N-terminal. rHuman GM-CSF migrates as a broad band between 15 and 25 kDa due to post-translation modification, in particular glycosilation.

**p.I:** 6.21

**Purity:** > 97% by SDS-PAGE gel

**Animal Free**

**Endotoxin Level\*:** <0.04 EU / µg protein  
(LAL method)

**Sequence:**

HHHHHHHHHHHAPARSPSPSTQPWEHVNAIQEARRLLNLSRDTAEE  
MNETVEVISEMFDLQEP TCLQTRLELYKQGLRGSLTKLKGPLTMMAS  
HYKQHCPTPETSCATQIITFESFKENLKDFLLVIPFDCWEPVQE

**Description:**

GMCSF is a cytokine that stimulates the growth and differentiation of hematopoietic precursor cells from various lineages, including granulocytes, macrophages, eosinophils and erythrocytes. It's involved in differentiation of dendritic cells and is a key factor in differentiation pathways leading form stem cells. GMCSF is produced by several cell types as monocytes, fibroblasts, endothelial cells and T- Lymphocytes in response to a number of inflammatory mediators present in the hemopoietic environment and peripheral sites of inflammation. Human GMCF is an important therapeutic cytokine used in the treatment of myeloid leukemia, neutropenia and aplastic anemia and it could also become interesting in treatments following bone marrow transplantation. Its biological activity is performed by binding to a specific receptor complex which is composed of a cytokine-specific alpha chain and B chain shared with the receptors for interleukin-3 and interleukin-5. GMCSR has been identified to mediate in the activation of Jak-Stat and MAPK pathways.

**Source:**

Produced by transient expression of human recombinant Granulocyte-macrophage colony-stimulating factor (GM-CSF) in non-transgenic plants. GM-CSF contains a 10-His-tag at the N-terminal end and is purified by sequential chromatography (Affinity and Anionic exchange - FPLC). Contains no animal-derived components or impurities.

**Formulation:**

Recombinan human GM-CSF is lyophilized from 10 mM PBS buffer pH 7.6 and 0.2 M NaCl.

**Reconstitution Recommendation:**

Lyophilized protein should be reconstituted in water to a concentration of 25-50 ng / µl.

**Storage and Stability:**

This lyophilized preparation is stable at 2-8° C for short term, long storage it should be kept at -20°C. Reconstituted rh GM-CSF should be stored in working aliquots at -20°C. Repeated freezing and thawing is not recommended.

**Purity Confirmation:**

The protein was resolved by SDS polyacrylamide gel electrophoresis and the gel was stained with coomassie blue.

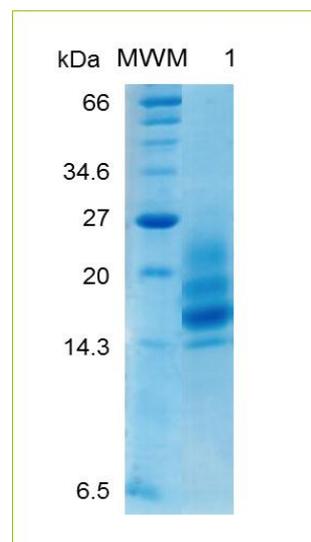


Fig 1. SDS-PAGE analysis of recombinant GM-CSF. Samples were loaded in 15% SDS-polyacrylamide gel and stained with Coomassie blue. Lane 1: Molecular weight marker (kDa); lane 3- 5 contains 50, 250 and 500 ng of rhuman GM-CSF.

**We recommend for optimal usage follow instructions of batch Quality Control sheet**

**For R+D purposes only. Purchaser must determine the suitability of the product(s) for their particular use.**

## Recombinant human GM-CSF(Granulocyte-macrophage colony-stimulating factor )

### Serological Identification:

The protein was electrophoresed under reducing condition on a 15% SDS-polyacrylamide gel, transferred by electroblotting to a NC membrane and visualized by immune-detection with specific GM-CSF antibody.

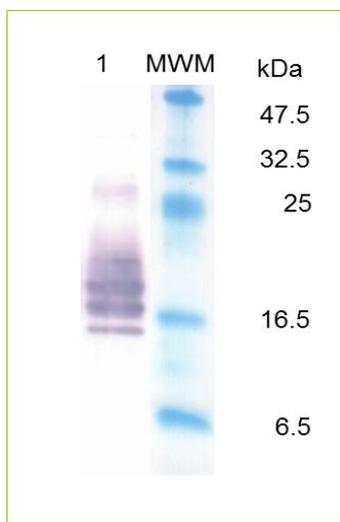
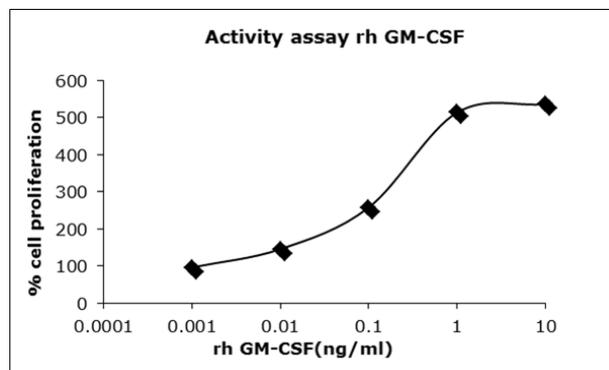


Figure 2. Analysis of recombinant GM-CSF with specific anti-human GM-CSF by Western Blot; lane 1: Molecular weight marker (kDa); lane 3- 5 contains 50, 250 and 500 ng of rhuman GM-CSF . All bands shown in lane 3-5 have been identify by MALDI-TOFF as recombinant GM-CSF.

### Biological Activity:

The activity of recombinant human GM-CSF is determined by the dose-dependent induction of human TF-1 proliferation cells. \*Cell proliferation was measured by MTT method.

ED50 is  $\leq 0.05$  ng/mL.



### References

- Kitamura, T. et al., 1989. Establishment and characterization of a unique human cell line that proliferates dependently on GM-CSF, IL-3 or erythropoietin. *J. Cell Physiol.*, 140 (3 ): 323-334.
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- Stout B. A. et al., 2004. IL-5 and granulocyte-macrophage colony stimulating factor activate STAT 3 and STAT 5 and promote Pim-1 and cyclin D3 protein expression in human eosinophils. *J. Immunol.*, Nov; 15 173 (10): 6409-6417.
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\*Agrenvec products are expressed in a plant system and intrinsically have extremely low endotoxin levels and are Animal-free