# Human granulocyte colony stimulating factor (hG-CSF): cloning and expression in *E. coli* cells

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**Saber M. A. [*], Moubasher M. H. [**], Shaheen M. M. [*], Mamdouh S. [*] and Omar H. A. [*]**
[*]Biochemistry and molecular Biology Department, Theodor Bilharz Research Institute, Giza – Egypt.
[**] Botany and Microbiology Department, Faculty of Science, Cairo University, Egypt.

## ABSTRACT

*The human granulocyte colony-stimulating factor (hG-CSF) is a growth factor or cytokine produced by a number of different tissues to stimulate the bone marrow for granulocytes and stem cells production. In this study, the hG-CSF mRNA was isolated from human peripheral blood mononuclear cells (PBMCs) cultured in-vitro and induced by lipo-polysaccharide. The cDNA of hG-CSF was amplified by PCR followed by DNA sequencing. Sequence analysis using NCBI Blast program revealed a 100% homology to the human G-CSF. The hG-CSF cDNA fragment was inserted into pET-3a expression vector and transformed into TOP-10 competent E. coli cells. The recombinant pET-3a/hG-CSF plasmid was transformed into different strains of BL21 E. coli cells. It was successfully expressed in BL21-Gold (DE3) strain. Western blotting of recombinant bacterial lysate using anti-human G-CSF showed a positive band at the MW of ~19 kDa. This confirms that the expressed protein has the same molecular weight and the immunogenicity against the specific antibodies of hG-CSF. Results proved that the E. coli strain BL21-Gold (DE3) carrying the construct pET-3a/hG-CSF is expressing rhG-CSF protein efficiently and can be used for scaling-up production.*

***Keywords:*** *Granulocyte colony-stimulating factor (G-CSF), Growth factor, pET-3a expression system, Cloning, Gene expression.*

## INTRODUCTION

The granulocyte colony-stimulating factor (G-CSF) is a hematopoietic growth factor that regulates proliferation and differentiation of neutrophil precursor cells (Nagata, 1989).The G-CSF is useful in the treatment of a wide range of complex disorders, including leucopenia and acquired immune deficiency syndrome, (AIDS) (Heit *et al.,* 2006), myelodysplastic syndrome (MDS), H1N1 influenza (Huang *et al.*, 2010), pulmonary alveolar proteinosis (Tazawa *et al.,* 2010), epilepsy (Zhang *et al.,* 2010), leukemia (Beekman and Touw, 2010), cancer (Gascon *et al.*, 2010), myocardial infarction (Louzada *et al.*, 2010), cardiomyopathy (Macambira *et al.*, 2009) and bone marrow transplantation (Kim *et al.*, 2009; Alrokayan, 2011).

The human G-CSF gene exists on chromosome 17 in region q21-q22 and consists of five exons and four introns of about 2500 nucleotides (Nagata *et al.,* 1986). Two different human G-CSF mRNAs are generated by alternative splicing of the introns into two translated isoforms G-CSFa and G-CSFb. The G-CSFa and G-CSFb have 30 amino acid signal peptides for secretion of G-CSF in soluble form.