

# **Application of Biotechnology for Production of Recombinant Protein (Human Interferon Gamma)**

Thesis

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fulfillment of M.Sc.*

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## **Declaration**

*I declare that this thesis has been composed by myself and that work of which it is a record has been done by myself. It has not been submitted for a degree at this or any other university.*

*Hend Okasha Ahmed Ali*



***TO THE SOUL OF MY FATHER***

***TO MY GREAT MOTHER***

***TO WHOM I OWED MY DEEPEST GRATITUDE***

***MY SISTER***

***MY UNCLES***

***MY FRIENDS***

***MY DEAR HUSBAND***

***&***

***MY LOVELY DAUGHTER***

***"Mariam"***

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# **ABSTRACT**

Interferon gamma (IFN- $\gamma$ ), also called immune interferon, is chemically and pharmacologically distinct from IFN- $\alpha$  and IFN- $\beta$ . It has weaker antiviral activity, more potent immunomodulator properties including macrophage activation, produced mainly by activated T-cells and natural killer.

IFN- $\gamma$  is a drug used to reduce the frequency and severity of serious infections associated with chronic granulomatous disease (CGD) and delays the effects of osteopetrosis in patients with severe malignant forms. IFN- $\gamma$  is currently in late stage of clinical trials in treatment of HCV and Leshmaniasis.

In this study hIFN- $\gamma$  was induced and isolated from human Peripheral blood mononuclear cells (PBMC's), analyzed by digestion, sequencing and cloned in pCRII TOPO TA cloning vector to maintain its integrity.

The hIFN- $\gamma$  ORF was sub-cloned in pET-15b expression vector and analyzed by PCR. The recombinant pET-15b/hIFN- $\gamma$  vector was transformed into several expression bacterial strains and was successfully expressed using Isopropylthio -D-galactoside (IPTG) as an inducer in Rosetta (DE3) bacterial strain and tested by Western blot.

The results of this work indicate that the expressed recombinant protein is the hIFN- $\gamma$  protein which confirmed by the molecular weight and the immunogenicity against the hIFN- $\gamma$  antibodies.

## **LIST OF ABBREVIATIONS**

<b>a.a</b>	Amino acid
<b>Ab</b>	Antibody
<b>AD</b>	Atopic dermatitis
<b>ampR</b>	Ampicillin resistance
<b>APCs</b>	Antigen presenting cells
<b>Arg</b>	Arginine
<b>Asp</b>	Aspartic acid
<b>AMV</b>	Avian myeloblastosis virus
<b>BCGF</b>	B-cell growth factor
<b>BHK</b>	Baby hamster kidney
<b>BLAST</b>	Basic Local Alignment Search Tool
<b>bp</b>	Base pair
<b>BSA</b>	Bovine serum albumin
<b>cDNA</b>	Complementary deoxyribonucleic acid
<b>cfu</b>	Colony forming per unit
<b>CGD</b>	Chronic granulomatous disease
<b>CHO</b>	Chinese hamster ovary
<b>Claudin1</b>	CLDN1
<b>CMV</b>	Cytomegalovirus
<b>DAB</b>	3, 3'-Diaminobenzidine
<b>DEPC</b>	Diethylpyrocarbonate
<b>DNA</b>	Deoxribonucleic acid
<b>dNTP</b>	Deoxy nucleotide tri-phosphate



<b><i>E. coli</i></b>	<i>Escherichia coli</i>
<b>FDA</b>	Food and drug administration
<b>EDTA</b>	Ethyline diamine tetraacetate
<b>FBSA</b>	Fetal bovine serum albumin
<b>FCS</b>	Fetal calf serum
<b>GAF</b>	Gamma interferon activator factor
<b>GAS</b>	Gamma interferon activation site
<b>Gln</b>	Glutamine
<b>Glu</b>	Glutamic acid
<b>Gly</b>	Glycine
<b>HBsAg</b>	Hepatitis B surface antigen
<b>HGH</b>	Human growth hormone
<b>hIFN-<math>\gamma</math></b>	Human interferon gamma
<b>IBs</b>	Inclusion bodies
<b>IFN-<math>\alpha</math></b>	Interferon alpha
<b>IFN-<math>\beta</math></b>	Interferon beta
<b>IFN-<math>\gamma</math>R</b>	Interferon gamma receptor
<b>IFN-<math>\omega</math></b>	Interferon omega
<b>IFN-<math>\tau</math></b>	Interferon tau
<b>IgE</b>	Immunoglobulin E
<b>IgG</b>	Immunoglobulin G
<b>IL</b>	Interleukin
<b>Ile</b>	Isoleucine
<b>IPTG</b>	Isopropylthio -D-galactoside
<b>IRF</b>	Interferon transcription factor
<b>ISGF</b>	Interferon stimulated gene factor

<b>ISRE</b>	Interferon stimulated response element
<b>JAK</b>	Janus family kinase
<b>KDa</b>	Kilo-dalton
<b>lac</b>	Lactose utilization operon
<b>LB medium</b>	Luria-Bertani medium
<b>Leu</b>	Leucine
<b>Lys</b>	Lysine
<b>MCS</b>	Multiple cloning site
<b>MHC</b>	Major histocompatibility complex
<b>MNC</b>	Mononuclear cells
<b>MOPS</b>	3-[N-morpholino]propanesulfonic acid
<b>mRNA</b>	Messenger Ribonucleic Acid
<b>MW</b>	Molecular weight
<b>NCBI</b>	National Center for Biotechnology Information
<b>NK</b>	Natural killers
<b>O.D</b>	Optical density
<b>ORF</b>	Open reading frame
<b>Ori</b>	Origin of replication
<b>OPC</b>	Oropharyngeal candidiasis
<b>PAGE</b>	Polyacrylamide gel electrophoresis
<b>PBMCs</b>	Peripheral blood mononuclear cells
<b>PCR</b>	Polymerase chain reaction
<b>PEG</b>	Polyethylene glycol
<b>PHA</b>	Phytohemagglutinin
<b>PGK</b>	3phosphoglycerate kinase promoter
<b>pL</b>	Phage lambda promoter

<b>PMN or PML</b>	Polymorphonuclear leukocytes
<b>PMSF</b>	Phenyl methyl sulfoxide
<b>PPD</b>	Purified protein derivatives
<b>Pro</b>	Proline
<b>PSI-BLAST</b>	Position-specific iterated BLAST
<b>PTPs</b>	Protein tyrosine phosphatases
<b>PVDF</b>	Polyvinylidene flouride
<b>RBS</b>	Ribosomal binding site
<b>RNA</b>	Ribonucleic acid
<b>rpm</b>	Round per minute
<b>RPMI</b>	Roswell park Memorial institute
<b>RT-PCR</b>	Reverse transcription PCR
<b><i>S. cerevisiae</i></b>	<i>Saccharomyces cerevisiae</i>
<b>SDS</b>	Sodium dodecyl sulfate
<b>SEA</b>	Staphylococcus enterotoxin A
<b>SEB</b>	Staphylococcus enterotoxin B
<b>SOC</b>	Super optimal catabolic repression medium
<b>Stat</b>	Signal transducer and activator of transcription
<b>STD</b>	Standard for DNA
<b>Ta</b>	Annealing temperature
<b>TAE</b>	Tris-acetate DNA electrode buffer
<b>TE</b>	Tris EDTA buffer
<b>TEMED</b>	N, N, N', N' –tetra-methylenediamine
<b>Th</b>	T helper cell
<b><i>T<sub>m</sub></i></b>	Melting temperature

<b>TNF</b>	Tumor necrosis factor
<b>TPA</b>	Tissue plasminogene activator
<b>tRNA</b>	Transfer Ribonucleic Acid

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