



Ain Shams University  
Faculty of Science

# *Biochemical Studies on Recombinant Interferon Gamma Produced in E. coli*

*Ph.D. thesis*

Submitted to Faculty of Science  
Ain Shams University

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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 SOULS OF MY FATHER, MY UNCLE,  
AND MY DEAR GRAND MOTHER***

***TO MY GREAT MOTHER***

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

***MY SISTER***

***MY FRIENDS***

***MY DEAR HUSBAND***

***MY LOVELY DAUGHTER***

***"MARIAM"***

***&***

***MY SWEET HEART SON***

***"YASEEN"***

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## **ARABIC ABSTRACT**

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***Hend Okasha***



# **ABSTRACT**

Production of therapeutic proteins in prokaryotic system, *Escherichia coli* (*E.coli*); has been recognized as an effective stage for production of recombinant human interferon gamma (rhIFN- $\gamma$ ). Modification of rhIFN- $\gamma$  expression is a line that can be positively employed for increasing the yield of production through optimization of induction conditions in shaker flasks to be applied onto batch culture of recombinant *E. coli*. Hence, in this research induction conditions for the over-production of rhIFN- $\gamma$  including type of media, pH, type and amount of inducer were optimized. The factors considered for optimized conditions of the recombinant *E.coli* were the use of growth medium LB, neutral pH7 and the inducer (lactose) at final concentration 2mM. These factors found to be useful in batch process development. The cell density was reached to 7gm/L wet cell weight after 12h of batch fermentation.

Commonly, the recombinant proteins were produced in *E. coli* as insoluble aggregates called inclusion bodies (IBs). A method for purification and refolding of rhIFN- $\gamma$  from IBs has been designated. It includes solubilization of IBs in 8M guanidinium hydrochloride; refolding of rhIFN- $\gamma$  by rapid dilution method; and protein purification by Hitrap Q XL strong anion chromatography. The rhIFN- $\gamma$  obtained has been characterized by immunogenicity against the hIFN- $\gamma$  antibodies. The specific activity of purified rhIFN- $\gamma$  was  $1.87 \times 10^7$  IU/mg compared to standard rhIFN- $\gamma$  via new MxA reporter gene assay which depends on identifying MxA mRNA level using real time PCR. The rhIFN- $\gamma$  increases expression of the MxA gene product in direct relation to the dose of rhIFN- $\gamma$  in IU.

# **LIST OF ABBREVIATIONS**

|             |                                      |
|-------------|--------------------------------------|
| <b>a.a</b>  | Amino acid                           |
| <b>Ab</b>   | Antibody                             |
| <b>AC</b>   | Affinity chromatography              |
| <b>Amp</b>  | Ampicillin                           |
| <b>APCs</b> | Antigen presenting cells             |
| <b>Arg</b>  | Arginine                             |
| <b>Asp</b>  | Aspartic acid                        |
| <b>BCGF</b> | B-cell growth factor                 |
| <b>BCIP</b> | 5-bromo-4-chloro-3-indolyl phosphate |
| <b>bp</b>   | Base pair                            |
| <b>BSA</b>  | Bovine serum albumin                 |
| <b>cDNA</b> | Complementary deoxyribonucleic acid  |
| <b>CGD</b>  | Chronic granulomatous disease        |
| <b>Cham</b> | Chloramphenicol                      |
| <b>CPE</b>  | Cytopathic effect                    |
| <b>CPE</b>  | Cytopathic effect inhibition assay   |
| <b>Ct</b>   | Cycle threshold                      |

|                                |  |
|--------------------------------|--|
| <b>DF</b>                      | Dilution factor                        |
| <b>DNA</b>                     | Deoxribonucleic acid                   |
| <b>dNTP</b>                    | Deoxy nucleotide tri-phosphate         |
| <b>DTT</b>                     | Dithiothritol                          |
| <b><i>E.coli</i></b>           | <i>Escherichia coli</i>                |
| <b>EDTA</b>                    | Ethyline diamine tetraacetate          |
| <b>EIA</b>                     | enzyme immunoassay                     |
| <b>FBSA</b>                    | Fetal bovine serum albumin             |
| <b>FDA</b>                     | Food and drug administration           |
| <b>FPLC</b>                    | Fast protein liquid chromatography     |
| <b>GdmCl</b>                   | Guanidinium hydrochloride              |
| <b>Gln</b>                     | Glutamine                              |
| <b>Glu</b>                     | Glutamic acid                          |
| <b>Gly</b>                     | Glycine                                |
| <b>GuSCN</b>                   | Guanidinium thiocyanate                |
| <b>h</b>                       | Hour                                   |
| <b>HIC</b>                     | Hydrophobic interaction chromatography |
| <b>HIV</b>                     | Human immunodeficiency virus           |
| <b>IBs</b>                     | Inclusion bodies                       |
| <b>IEC</b>                     | Ion exchange chromatography            |
| <b>IFN-<math>\alpha</math></b> | Interferon alpha                       |
| <b>IFN-<math>\beta</math></b>  | Interferon beta                        |

|                                |   |
|--------------------------------|---|
| <b>IFN-<math>\tau</math></b>   | Interferon tau                                      |
| <b>IFN-<math>\omega</math></b> | Interferon omega                                    |
| <b>IgG</b>                     | Immunoglobulin G                                    |
| <b>IL</b>                      | Interleukin   |
| <b>IPTG</b>                    | Isopropylthio -D-galactoside                        |
| <b>ISG</b>                     | Interferon stimulated gene                          |
| <b>IU</b>                      | International unit                                  |
| <b>JAK</b>                     | Janus 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>mAU</b>                     | Milli absorbance unit                               |
| <b>MCS</b>                     | Multiple cloning site                               |
| <b>MHC</b>                     | Major histocompatibility complex                    |
| <b>min</b>                     | minute  |
| <b>MMLV-RT</b>                 | moloney murine leukemia virus reverse transcriptase |
| <b>mRNA</b>                    | Messenger Ribonucleic Acid                          |
| <b>mSc/cm</b>                  | Milli Siemens per centimeter                        |

|                                  |  |
|----------------------------------|--|
| <b>Mwt</b>                       | Molecular weight                       |
| <b>MWCO</b>                      | Molecular weight cutoff                |
| <b>MxA</b>                       | Myxovirus resistance protein A         |
| <b>NBT</b>                       | Nito blue tetrazolium                  |
| <b>NK</b>                        | Natural killers                        |
| <b>OAS1</b>                      | 2'-5'-oligoadenylate synthetase 1      |
| <b>OD</b>                        | Optical density                        |
| <b>PAGE</b>                      | Polyacrylamide gel electrophoresis     |
| <b>PBMCs</b>                     | Peripheral blood mononuclear cells     |
| <b>PCR</b>                       | Polymerase chain reaction              |
| <b>PGK</b>                       | 3-phosphoglycerate kinase<br>promoter  |
| <b>pI</b>                        | Protein isoelectric point              |
| <b>PKR</b>                       | Protein kinase RNA activated           |
| <b>PMN</b>                       | Polymorphonuclear leukocytes           |
| <b>PMSF</b>                      | Phenyl methyl sulfoxide                |
| <b>pSS</b>                       | Primary Sjögren's syndrome             |
| <b>PTM</b>                       | Post transitional modification         |
| <b>qPCR</b>                      | Quantitative polymerase chain reaction |
| <b>RBS</b>                       | Ribosomal binding site                 |
| <b>rhIFN-<math>\gamma</math></b> | Recombinant human interferon gamma     |
| <b>RNA</b>                       | Ribonucleic acid                       |

|                |  |
|----------------|--|
| <b>RNase L</b> | Ribonuclease L                                   |
| <b>RPC</b>     | Reversed phase chromatography                    |
| <b>rpm</b>     | Round per minute                                 |
| <b>RPMI</b>    | Roswell park Memorial institute                  |
| <b>SB</b>      | Super broth                                      |
| <b>SDS</b>     | Sodium dodecyl sulfate                           |
| <b>SEA</b>     | Staphylococcus enterotoxin A                     |
| <b>SEB</b>     | Staphylococcus enterotoxin B                     |
| <b>SEC</b>     | Size exclusion chromatography                    |
| <b>SOB</b>     | Super optimal both                               |
| <b>STAT</b>    | signal transducer and activator of transcription |
| <b>TB</b>      | Terrific broth                                   |
| <b>TEMED</b>   | N, N, N', N' –tetra-methylenediamine             |
| <b>Th</b>      | T helper cell                                    |
| <b>TNF</b>     | Tumor necrosis factor                            |
| <b>tRNA</b>    | Transfer Ribonucleic Acid                        |
| <b>TY</b>      | Tryptone yeast extract media                     |
| <b>vvm</b>     | Vessel volume per minute                         |
| <b>WCW</b>     | Wet cell weight                                  |
| <b>WHO</b>     | world health organization                        |

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