# PRODUCTION OF RECOMBINANT HUMAN INTERFERON-GAMMA IN *Escherichia coli*

**Mussakhmetov A., Turgimbayeva A., Khassenov B.**

*National center for biotechnology*
*13/5,Korgalzhyn road, Astana, Z05K8D5, Kazakhstan*
*musakhmetov@mail.ru*

**ABSTRACT**

**Human interferon-gamma (IFN-γ) is an important immune component that protects against pathogens and tumours. The IFN-γ cytokine, the only member of the type II interferon family, is produced predominantly by natural killer (NK) and natural killer T (NKT) cells as part of the innate immune response, and by Th1 CD4 and CD8 cytotoxic T lymphocyte (CTL) effector T cells upon the development of antigen-specific immunity. The recombinant production of IFN-γ has significant practical value. Here, we describe the production of recombinant human IFN-γ, the gene of which (hIFNG) was efficiently expressed in *E. coli.* The hIFNG gene was assembled from synthetic fragments, amplified by PCR, and subsequently cloned into several plasmid vectors. The target gene was cloned into recombinant vectors both with and without a hexa-histidine tag. The *Rosetta2 (DE3) E. coli* strain was used as the host strain for recombinant protein expression and high levels of synthesis of the recombinant human IFN-γ were achieved by the optimisation of induction conditions. The optimal concentration of isopropyl-β-D-thiogalactopyranoside (IPTG) was found to be 0.005 mM. The recombinant protein was shown to accumulate in the E. coli cells as inclusion bodies, and highly pure recombinant human IFN-γ was obtained by multi-stage extraction with chaotropic agents. Purification was carried out by a combination of anion and cation exchange chromatography using Q- and SP-sepharose.**

**Keywords: Interferon-gamma, cytokine, recombinant protein**

## INTRODUCTION

It is known that gamma-interferon (IFN-γ)is not associated with the type-I interferons by both genetic relatedness or by the amino acid sequence [1,2]. Human interferon-gamma gene is located on the 12th chromosome [3]. The expression of the interferon is induced by a unique set of stimulus and is produced by T-lymphocytes and NK cells.The *hifn-γ* gene encodes a 143 amino-acid residue long polypeptide and contains two sites of glycosylation: $Asn^{25}$ and $Asn^{97}$. The polypeptide chain is sated with the lysine and arginine residue, and the N-terminal sequence of natural hIFN-γ is pyroglutamic acid, instead of usual methionine [4].

Interferon-gamma has a structure of a glycosylated homodimer. Nevertheless, in comparison with native interferon-gamma,non-glycosylated recombinant interferon-gamma, produced in bacteria, maintainsdimeric conformation and biological activity [5].

Interferon-gamma is pleiotropic cytokine, playing crucial role in the innate and acquired immunity. It influences on antiviral and antibacterial protection of an organism, regulation of a cellular cycle (apoptosis) and participates in the inflammatory process [6].

The first expression of recombinant interferon-gamma in *E.coli* cells was carried out in 1982 [7]. Then it was found that rIFN-γ is accumulated in *E.coli* cells in the inclusion bodies.Extraction of protein from inclusion bodiesinvolves additional experimental steps for a protein refolding to obtain correct conformation [8].

To obtain the protein in water-soluble fraction various types of cells were tested: mammalian cells, cells of *Bacillus subtilis* bacterium and *Pichiapastoris* yeast cells. The main problem of these systems was low level of expression and a long time of cultivation.These drawbacks forced to use *E.coli* cells as the main expression system [9].

The analysis of literature data showed that despite distinctions in certainsteps, procedure for obtaining of pure and biologically active human interferon-gamma in *E.coli* cells, consists of the following steps: accumulation of recombinant IFN-γ in the form of inclusion bodies; dissolving and denaturation of proteins from inclusion bodies in high-molar solutions of guanidine hydrochloride or urea; purification of the denatured protein by chromatography methods; refolding and concentrationof target protein [10].It was noted that after the concentration, recombinant interferon-gamma aggregatedthatcanlead to decrease of biological activity [11]. An important element of the strategy is selection and optimization of extraction conditions and purification of recombinant protein.

The aim of this work was to develop technology for producingthe recombinant human interferon-gamma in*E.coli* cells. To achieve this aim the following tasks were set: design of an effective expression vector for synthesis ofhuman interferon-gamma in bacteria; obtaining of bacterial strain-producer of recombinant human interferon-gamma; development of extraction procedure and purification of recombinant human interferon-gamma.

## MATERIALS AND METHODS

### *Strains, vectors and oligonucleotides*

JM109 and DH5α strains were used for preparative plasmid DNA isolation and vector deign respectively. Rosetta2(DE3) and BL21(DE3) strains were used as host for recombinant IFN-γ expression.

The following plasmid vectors were used: pGEM-T (Promega), pET-9a, pET-11aand pET-28c(+) (Novagen).

Used oligonucleotides arepresentedinthe table 1.

**Table 1**. Oligonucleotides

| Name | Sequence |
|---|---|
| InfG1 | ATGCAGGACCCGTACGTAAAAGAGGCCGAAAATTTGAAAAAATATTTCAACGCTGGCCAT |
| InfG2 | GAATACCCAGGAAAAGAGTCCCGTTATCCGCTACATCAGAATGGCCAGCGTTGAAATATT |
| InfG3 | GACTCTTTTCCTGGGTATTCTGAAAAATTGGAAAGAGGAGTCTGATCGTAAAATCATGCA |
| InfG4 | ATTTTTGAACAGCTTAAAATAGAAGCTCACGATCTGGCTCTGCATGATTTTACGATCAGA |
| InfG5 | ATTTTAAGCTGTTCAAAAATTTCAAAGATGATCAGTCCATCCAGAAAAGCGTCGAGACGA |
| InfG6 | TTATTGCTATTGAAAAATTTCACGTTCATATCCTCTTTAATCGTCTCGACGCTTTTCTGG |

| | |
|---|---|
| InfG7 | AAATTTTTCAATAGCAATAAAAAAAAGCGTGACGACTTTGAGAAACTGACCAACTA CAGT |
| InfG8 | GCTCATGGATTGCTTTACGTTGTACATTCAGGTCCGTCACACTGTAGTTGGTCAGTTT CT |
| InfG9 | ACGTAAAGCAATCCATGAGCTGATCCAGGTGATGGCGGAACTGAGTCCTGCGGCCA AGAC |
| InfG10 | ACGCCCGCGGAACAGCATTTGGCTACGCTTACGTTTACCCGTCTTGGCCGCAGGACT CAG |
| InfG11 | AAATGCTGTTCCGCGGGCGTCGTGCAAGCCAGTAATAA |
| InfGfw | ATGCAGGACCCGTACGTAAAA |
| InfGrv | TTATTACTGGCTTGCACGACG |
| InfG_NdeI | GGGAATTCCATATGCAGGACCCGTACGTAAAA |
| InfG_BamHI | CGCGGATCCGCGTTATTACTGGCTTGCACGACG |
| InfG_NcoI | CATGCCATGGCAGGACCCGTACGTAAAA |
| hINFdGfw | AAGGAGATATACCATGCAGGACCCGTACGTAA |
| hINFdGrv | TTACGTACGGGTCCTGCATGGTATATCTCCTT |

***Media and reagents***

In thiswork Luria-Bertanibroth(1% tryptone, 0,5% yeast extract, 0,5% NaCl) and the SOC media (2%tryptone, 0,5% yeast extract, 0,05% NaCl, 2,5 mMKCl, 20 mM$MgSO_4$, 20mM glucose, pH 7,5) were used. Concentration of ampicillin and kanamycin in the medium was 150 μg/ml and 50 μg/ml respectively.

***Assembling of human interferon-gamma gene***

Assembling of the gene was carried out by the PCR *de novo*. Concentration of oligonucleotides (InfG1-InfG12) in initial solution was 100 pmol/μl. Firstlywe mixed 5 μl of each oligomer,then we did the PCR.ThePCR-mix included:oligomer mix, the dNTP mix (10 mM each), Phusion polymerase (1 unit),polymerase buffer (1X) with $MgCl_2$ (1,5 mM). In the second round,the final assembling of the gene was carried out with the PCR-product of the first round and flanking oligonucleotides (InfGfw/InfGrv).After PCR the human interferon-gamma gene was purified by chloroform-ethanol precipitation and cloned intopGEM-T (Promega) plasmid vector. The cloning was accomplishedaccording to the manufacturer's protocol. Plasmid DNA was isolated by the MiniPrepkit (Promega, the USA), according to the manufacturer's protocol.

***Cloning of ifn-γ gene into expression vectors***

Restriction of plasmid DNA was carried out with enzymes: *Nde*I, *BamH*I and *Nco*Iin 2X Tango buffer.Ligation was accomplished by T4 ligase and T4 Ligase Buffer (Thermo Scientific). Ligation was performed at +4°C, 16 hours. Water bath and the solid-state thermostat were used for maintenance of the corresponding temperature conditions. All manipulationsof plasmid vectors design were carried out under described protocols [12].

The *ifn-γ* gene was amplified from pGEM-T/IFN-γ vector with primers (InfG_NdeI/InfG_BamHI) and cloned into vectors pET-9a, pET-11a and pET-28c(+) between*Nde*I and *BamH*I sites. In addition, the *ifn-γ* gene was amplified from pGEM-T/IFN-γ vector bycorresponding primers (InfG_NcoI/InfG_BamHI) and was cloned into pET-28c(+) vector.Then, unnecessary guanine in the fourth position at the beginning of ORF (open reading frame) was removed by site-directed mutagenesis. This procedure was accomplished by protocol described in [13]. At the first stage of PCRwe used couple of primers (hIFNdGfw/hIFNdGrv) and pET-28c (+)/IFN-γ vector in which the gene of interferon-gamma was cloned into *Nco*I and *BamH*I sites.Reaction conditions: the initial denaturation at +98°C during 30 seconds; +98°C - 10 seconds, +58°C – 30 seconds, 72°C – 3 minutes (30 cycles); final elongation 72°C – 10 minutes and 10 minutes at +10°C. The obtained PCR product was treated by *Dpn*Irestrictase (Thermo scientific) in the 1X Tango buffer for degradation of initial DNA. Hydrolysis/degradation of methylated DNA was carried out at +37°C during 2 hours with the subsequent inactivation of theenzyme at +80°C for 20 minutes. Competent cells were transformed with product of reaction after ethanol precipitation. After that, plasmid DNA was isolated from the cells and sequenced.

***Sequencing***

Nucleotide sequence determination was carried out by Sanger's method (BigDye Terminator 3.1 Cycle sequencing Kit).

Purification of reaction mix from contaminants was accomplished by acetate-ethanol mix. Separation of gene fragments was done by the automatic ABI 3730xl sequencer (Applied Biosystems, the USA). The analysis of chromatograms and comparison with reference sequence was carried out by the Vector NTI (version 11) software package.

***Analytical procedures***

Protein concentration was measured by Bradford method [14].SDS-PAGE analysis was performed according to Laemmli using 12% polyacrylamide gels [15].Bacterial lipopolysaccharide in protein sample was analysed by silver staining technique in polyacrylamide gel [16].

## RESULTS AND DISCUSSION

HIFN-γ gene from GenBank (NCBI Reference Sequence: NM_000619.2) data was used as reference sequence. This sequence was codon-optimized for expression in *E. coli* cells. According to this sequence, oligonucleotides were synthesized for designing full-size human interferon-gammagene(489 b.p.). The final PCR product is presented in figure 1. After purifying and precipitation, concentration of the sample was265 ng/μl.

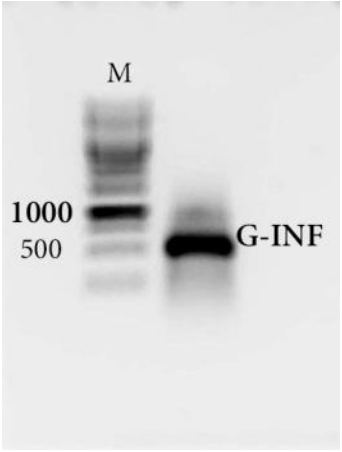
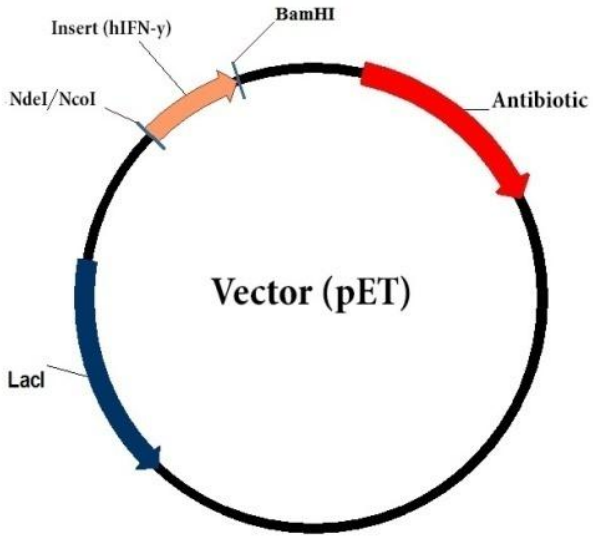



**Fig. 1.**Result of PCR-assembling of human interferon-gamma gene

After cloning of hIFN-γ gene inpGEM-T plasmid positive clones were selected by blue-white screening method and PCR-screening forM13 region. Sequencing of the hIFN-γ gene did not show any changes in nucleotide sequence of the gene. Two resultant expression vectors were named aspET28c(+)/6His-IFN-γ and pET-11a/IFN-γ (figure 2).

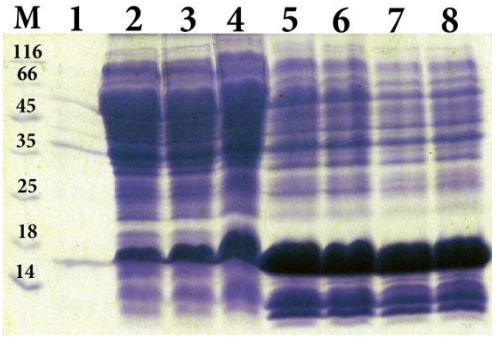



Antibiotic – ampicillin for a pET-11a/hIFN-γ vector and kanamycin for pET-28c(+)/6His-hIFN-γ and pET-28c(+)/hIFN-γ vectors

**Fig. 2.**The map of vectors for expression of hIFN-γ gene in *E.coli*

In the first recombinant vector (pET-28c(+)/6His-hIFN-γ) final protein has a N-terminal hexa-histidine tag, the protein from the second recombinant vector(pET-11a/hIFN-γ) doesn't have any additional polypeptides and completely corresponds to the human interferon-gamma (GenBank, CAA44325.1).

These vectors were transformed to the BL21(DE3) and Rosetta2(DE3) cells. Determination of expression level showed that the most effective is the Rosetta2(DE3) strain transformed with pET-28c(+)/6His-hIFN-γ vector. When we used pET-11a/hIFN-γ vector expression level was much lower than after using pET-28c(+)/6His-hIFN-γ.As an alternative to this vector for producinguntagged protein, the *hifn-γ* gene was cloned into pET-9a vector, however the level of expression of untagged recombinant protein was also lower than those obtained from pET-28c(+)/6His-hIFN-γ vector. The next strategy was to obtain untagged hIFN-γprotein,thereforethe pET-28c(+) vector with deleting $His_6$-tag region was used. The gene was cloned into *Nco*I and *BamH*I sites with the subsequent removal of a guanine in the fourth position by the site-directed mutagenesis. After transformation of Rosetta2(DE3) cells with new assembled plasmid vector, the level of expression of hIFN-γ protein was the same as 6His-hIFN-γ. Experiments on optimization of induction conditions of recombinant protein showed that the recombinant strain provide the high level of protein expression even at low concentration of inductor (isopropyl β-D-1-thiogalactopyranoside) (0,005 mM) in comparison with other works [9, 10, 17, 18].




M – protein ladder (14 kDa-116 kDa); 1 – not induced culture; 2 – IPTG 0,0005 mM; 3 – IPTG 0,001mM; 4 – IPTG 0,003mM; 5 – IPTG 0,005mM; 6 – IPTG 0,01mM; 7 – IPTG 0,1 mM; 8 – IPTG 0,3 mM

**Fig. 3.**Expression of hIFN-γ depending on IPTG concentration

According to results, our final expression vector and strains-producer have advantages than those described in literature (table 2).

**Table 2.**Comparison of our conditions of hIFN-γ expression with other authors

| Authors | Bacterial strain | Base expression vector | IPTG concentration |
|---|---|---|---|
| R. Khalilzadeh et al. [10] | BL21(DE3) | pET-3a | 3 mM |
| Fenfen Zhu et al. [17] | BL21(DE3) | pSUMO | 1 mM |
| Xiao Yan et al. [9] | BL21(DE3) | pET-28a(+) | 0.2 mM |
| M. Tileva et al. [18] | BL21(DE3) | pET-28a(+) | 0.1 mM |
| Our results | Rosetta2(DE3) | pET-28c(+) | 0.005 mM |

Extraction and purification of the recombinant hIFN-γ protein from inclusion bodies was accomplished by the multistage procedure, scheme is given in figure 4.

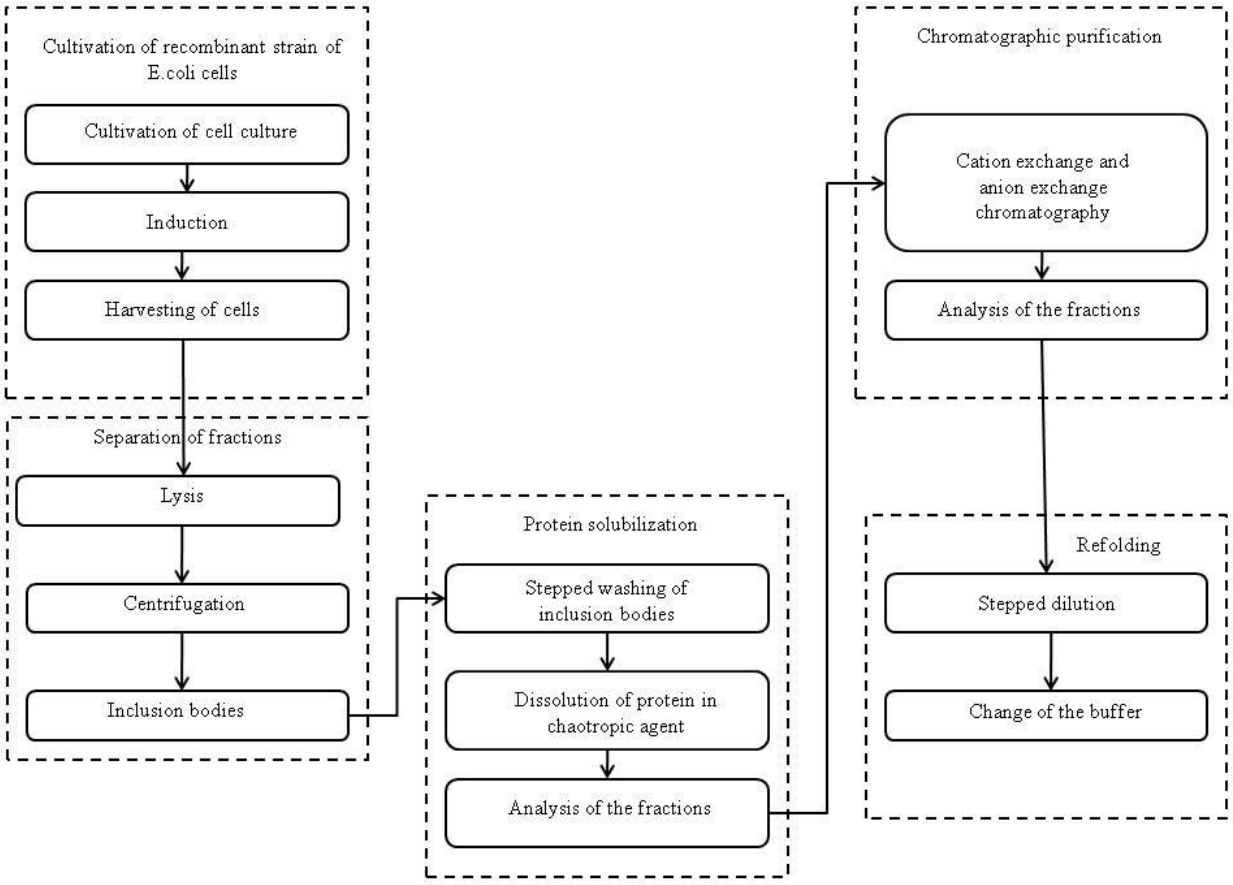



**Fig. 4.** Purification of recombinant hIFN-γ

The process of purification includes the following steps: cultivation of a recombinant Rosetta2(DE3)/pGIFNh cells; separation of a lysate in water-soluble and water-insoluble fractions; converting of protein from water-insoluble fraction to soluble state; chromatographic purification and refolding of recombinant protein.

It was established that recombinant human interferon-gamma in Rosetta2(DE3)/pGIFNh cells was accumulated as inclusion bodies with forming of aggregates. The induced cells were harvested by centrifugation at 6000 g for 7 min at +4°C. Pellet was resuspended in buffer 1:5 (w/v) (20 mMTris-HCl, pH 7,5, 20 mMNaCl). Then cells were lysed by ultrasonic sonication in the pulsed mode for 40 min.Extraction of inclusion bodies from the total bacterial lysate was carried out by the high-speed centrifugation.Full precipitation of the aggregated recombinant human interferon-gamma was observed during centrifugation within an hour at 18000g.

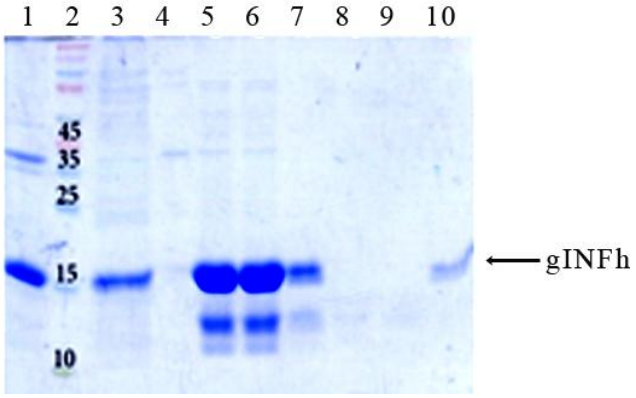
The recombinant human interferon-gamma was presented in water-insoluble form in inclusion bodies.Forconvertingthe protein to soluble state a guanidine-hydrochloride and urea were used. A prewashing of inclusion bodies was accomplished (for obtaining more pure recombinant protein)using three buffers:I (100 mMTris-HCl (pH 8,0), 1mM EDTA (pH 8,0), 100 mMNaCl), II (100 mMTris-HCl (pH 8,0), 1mM EDTA (pH 8,0), 500 mMNaCl, 1% Triton X-100) and III (100 mMTris-HCl (pH 8,0), 1mM EDTA (pH 8,0), 250 mM sucrose).The protocol of washing included the following procedures: the pellet of lysate was resuspended in 20 ml of the buffer I and centrifuged at 18000 g, within 30 minutes at +4°C, supernatant was removed and pellet was resuspended in the buffer II, and centrifuged (18000 g, 30 minutes, +4°C) and, then, pellet again was washed out in the buffer III. After the third centrifugation the pellet was presented as pure inclusion bodies withoutany impurities (nucleic acids and cell debris).

To convert the recombinant human interferon-gamma to a water-soluble state, pellet was resuspended in 20 ml of buffer IV (4M urea, 4M guanidine-hydrochloride, 50 mM sodium phosphate (pH 8,0), 1 mM EDTA (pH 8,0). Suspension was incubated at +4°C over night (16 hours) with continuous mixing. After centrifugation (18000 g, 30 minutes, +4°C) supernatant has contained protein extract with the denatured recombinant human interferon-gamma.

Purification of recombinant protein was performed in two steps: at first step contaminant proteinswere removed from protein extract, the second step included extraction of recombinant interferon-gamma from protein extract. We used the combination of anion exchange and cation exchange chromatography for this procedure.

According to the literature, the isoelectric point (pI)of human interferon-gamma is 8,5-9,3 [19]. Therefore, at pH index lower than 8, interferon-gamma behaves as positively charged molecule and doesn't have affinity to strong

anion exchange sorbents such as Q-sepharose and, meanwhile, it interacts with negatively charged molecules of cation exchanger such as SP-sepharose.The protein extract was loaded on the HiTrap Q (GE) column which was equilibrated with buffer V (4M urea, 4M guanidine- hydrochloride, 50 mM of sodium phosphate (pH 8,0), 1 mM EDTA (pH 8,0). Eluate passed through the column was collected and loaded on HiTrap SP (GE) column equilibrated with buffer VI (6M urea, 50 mM ammonium acetate, 2 mM EDTA (pH 8,0).After loading the extract, the column was washed out with 5 ml of buffer VI to remove nonspecific bound proteins, after that concentration of ammonium acetate in buffer was increased to 200 mM and not target proteins were washed out, then by increasing concentration of ammonium acetate to 500 mM the target protein was eluted (figure 5).




1 – pellet; 2 – marker; 3 –Triton X-100 wash; 4 –Sucrose buffer wash; 5 – solubilization with 8 Murea; 6 – Flow from Q-sepharose; 7 – elution from Q-sepharose; 8 – Flow fromSP-sepharose; 9 – wash from SP-sepharose; 10 – elution from SP-sepharose

**Fig. 5.** Purification of recombinant human interferon-gamma

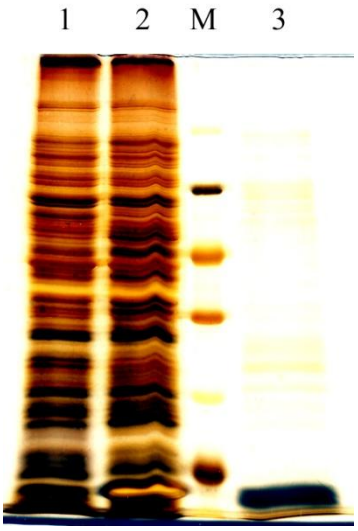
The correct conformation of protein molecule has a great importance and the tertiary structure of protein is the defining factor in the regulation of activity of interferons [20]. Using of chaotropic agents like urea and guanidine-hydrochloride leads to destruction of tertiary structure of the protein however the denaturation by chaotropic agents is reversible process.The refolding procedure included the following steps: gradual decrease of concentration of chaotropic agents and addition of the stabilizers interfering repeated aggregation of protein.Decrease of concentrationofdenaturing agents is carried out by a stepped dialysis that leads to dilution of protein. Therefore it is necessary, after dialysis, to concentrate the protein by chromatography column.

Procedure ofrefolding of recombinant human interferon-gamma was accomplished by simultaneous decrease of concentration of urea and guanidine-hydrochloride in the dialysis buffer to 2 M. This procedure was done by dialysis of the protein inthe dialysis bag (membrane pore:14 kDa, Sigma) at +4°C during 16 hours on a magnetic mixer against the buffer VII (3,8-2,0 M urea, 3,8-2,0 M guanidine-hydrochloride, 50 mM phosphate buffer, 5% glycerol, 5% sucrose and 1 mM EDTA, pH 7,5).Further decrease of urea concentration led to aggregation of interferon that was revealedbyturbidity of the sample. The stabilizing agents were added for maintenance of recombinant interferon-gamma in the dissolved state: L-arginine, L-proline, polyethyleneglycol (50 mMphosphate buffer, 5% sucrose, 0,05% PEG 3500, 500 mM L-arginine, 500 mM L-proline, pH 7,5).

The column chromatography was used for increase of protein concentration. The column was equilibrated with the buffer VIII (20 mMTris-HCl, 2 mM EDTA (pH 7,5), after that dialysed recombinant interferon was loaded on the HiTrap SP (GE) column. Washing of a column was accomplished with buffer IX (20 mMTris-HCl (pH 7,5), 200 mMNaCl, 2 mM EDTA). Elution of interferon was carried out with buffer X (20 mMTris-HCl (pH 7,5), 800 mMNaCl, 2 mM EDTA).

The following steps were the dialysis and change of the buffer. The dialysis bag and the 1X PBS buffer were usedin this process. The dialysis was performed during 20 hours at +4°C on a magnetic mixer with change of the buffer after 10 hours of a dialysis. At the end of the dialysis concentration of the samples was adjusted with PBS to 20 μg/ml, then it was aliquoted by 100 μland maintained on cryopreservation at -20°C.As a result of a refolding and a dialysis there were losses of interferon-gamma which constituted more than 50%.

Detection of bacterial endotoxin amount by silver staining technique showed high level of purity from endotoxins (figure 6).




1– BL21(DE3) lysate; 2– Arctic Exp.(DE3)RP/rhG-IFN lysate;3 – Recombinant hIFN-γ sample; M– Protein marker

**Fig. 6.** Silver staining of bacterial lysate and purified recombinant human interferon-gamma

Bioassay of the obtained interferon-gamma on BHK-21 cell culture confirmed/provedthat recombinant cytokine doesn't contain crucial concentration of endotoxins.

## CONCLUSION

Thus, the most effective recombinant vector for synthesis of recombinant human interferon-gamma in *E. coli* cells by plasmid expression is the pGIFNh based on vector pET-28c (+). Transformation by this vectorofRosetta2(DE3) cells resulted in production of the recombinant strain providing the high level of synthesis of protein (more than 40% of the total protein) at concentration of the inductor 0,005 mM.The procedure of purification of recombinant human interferon-gamma from inclusion bodies, providing the high level of purity of recombinant protein, is developed. In total, from 1 liter of culture 5,7 mg of the purified recombinant human interferon-gamma was obtained. For bioassay procedure the protein was put on cryopreservation.

Testing of recombinant interferon-gamma strain producer on pathogenicity at "Nutritest" LLP showed that Rosetta2 (DE3)/pGIFNh recombinant strain belongs to the 3rd class of danger. Strain producer of recombinant human interferon-gamma Rosetta2 (DE3)/pGIFNhwas deposited inthecollection of microorganisms of "Kazakh Research Institute of Food and Processing Industry" LLP under the number B-741.

## FUNDING

This work was performed in frame of Scientific Technical Program "Industrial Biotechnologies" on the project 03.12 "Development of the advanced production technology of recombinant human interferon-gamma with antiviral activity", financed by the Ministry of Education and Science of the Republic of Kazakhstan.

## Acknowledgment

We are grateful to the head of the laboratory of NCB, Ph.D.Shustov Alexander and researcher Kim Yury for advice and the help in accomplishment of experiments with cell culture.

## REFERENCES

1. Gray P.W., Leung, D.W., Pennica D. et al. Expression of human immune interferon cDNA in E. coli and monkey cells. *Nature*, 1982, vol. 295, pp. 503-508.

2. Gray P.W., Goeddel D.V. Structure of the human immune interferon gene.*Nature*, 1982,vol. 298, pp. 859-863.

3. DeGrado W.F., Wasserman Z.R.,Chowdhry V. Sequence and structural homologies among type I. *Annu. Rev. Immunol.*, 1993, vol. 11, pp. 571-611.

4. NargesMalek-Sabet, Mohammad Reza Masoumian, Majid Zeinali et al. Production, purification and chemical stability of recombinant human interferon-γ; in lowoxygentension condition: a formulation approach. *Preparative Biochemistry and Biotechnology*,2013.doi:10.1080/10826068.2012.762716.

5. Jan N.M.,Ijzermans and Richard L. Marquet. Interferon-gamma: A Review. *Immunobiol*, 1989, vol. 179, pp. 456-473.

6. Marina D. Kraaij, Elly J.F. Vereyken, Pieter J.M. et al. Human monocytes produce interferon-gamma upon stimulation with LPS.*Cytokine*, 2014, vol. 67, pp. 7-12.

7. Billiau A.,Heremans H., Vandekerckhove F. and Dillen C. Anti-interferon-gamma antibody protects mice against the generalized Shwartzman reaction.*Eur. J. Immuno*, 1987, vol. 17, pp. 1851.

8. Petrov S., Nacheva G., Ivanov I. Purification and refolding of recombinant human interferon-gamma in urea–ammonium chloride solution.*Protein Expression and Purification*,2010, vol. 73, pp. 70-73. doi:10.1016.

9. Xiao Yan & Sheng Hu & Yi-Xin Guan & Shan-Jing Yao.Coexpression of chaperonin GroEL/GroES markedly enhanced soluble and functional expression of recombinant human interferon-gamma in Escherichia coli. *ApplMicrobiolBiotechnol*, 2012, vol. 93, pp. 1065-1074. doi 10.1007/s00253-011-3599-2.

10**.** Khalilzadeh R., Shojaosadati S.A., N. Maghsoudiet al. Process development for production of recombinant human interferon-γexpressed in Escherichia coli.*J. Ind. MicrobiolBiotechnol*, 2004, vol. 31, pp. 63-69. doi10.1007/s10295-004-0117.

11. Hiroshi Yamaguchi and Masaya Miyazaki.Refolding Techniques for Recovering Biologically Active Recombinant Proteins from Inclusion Bodies.*Biomolecules*,2014, vol. 4, pp. 235-251. doi:10.3390/biom4010235.

12. S. Abeldenov, S. Kirillov, A. Nurmagambrtova, A. Kiribayeva, D. Silaev, B. Khassenov. Expression, purification and biochemical characterization of recombinant phosphohydrolaseAppa in Escherichia coli.*Biotechnology.Theory and practice*,2014, vol. 3, p. 61.

13. Yongzhen Xia, Wenqiao Chu, Qingsheng Qi and LuyingXun. New insights into the QuikChangeTM process guidethe use of Phusion DNA polymerase for site-directed mutagenesis.*Nucleic Acids Research*, 2015, vol. 43, no. 2.doi: 10.1093/nar/gku1189.

14. Bradford M.M. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding.*Anal.Biochem*., 1976, vol. 72, pp. 248-254.

15. Laemmli U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4.*Nature*, 1970, vol. 227, pp. 680-685.

16.Fomsgaard A., Freudenberg M.A., Galanos Ch. Modification of the silver staining technique to detect lipopolysaccharide in polyacrylamide gels.*Journal of clinical microbiology*, 1990,vol. 28, pp. 2627-2631.

17.Fenfen Zhu, Qi Wang, Hefang Pu et al. Optimization of soluble human interferon-γ production in Escherichia coli using SUMO fusion partner. *World J MicrobiolBiotechnol*, 2013, vol. 29, pp. 319-325. doi10.1007/s11274-012-1185-0.

18. Tileva M., Krachmarova E., Ivanov I.et al. Production of aggregation prone human interferon gamma and its mutant in highly soluble and biologically active form by SUMO fusion technology.*Protein Expression and Purification*,2016, vol. 117, pp. 26-34.

19. JafarMohammadian-Mosaabadi, HosseinNaderi-Manesh, Nader Maghsoudi et al. Improving purification of recombinant human interferon-γ expressed in *Escherichia coli*; eVect of removal of impurity on the process yield. *Protein Expression and Purification*, 2007, vol. 51, pp. 147-156.

20. Jamie R. Schoenbornand Christopher B. Wilson.Regulation of Interferon-gamma during innate and adaptive immune responses.*Advances in Immunology*, 2007, vol. 96.doi: 10.1016/S0065-2776(07)96002-2.

# *E. COLI* ЖАСУШАЛАРЫНДА РЕКОМБИНАНТТЫ АДАМ ГАММА-ИНТЕРФЕРОНЫН АЛУ

**Мусахметов А., Тұрғымбаева А., Хасенов Б.**

*Ұлттық биотехнология орталығы*
*Қорғалжын тас жолы, 13/5, Астана, Z05K8D5,Қазақстан*
*musakhmetov@mail.ru*

**ТҮЙІН**

**Адамның гамма-интерфероны жасушаішілік патогендер мен ісік жасушаларын бақылауда иммундық жүйенің маңызды бөлігі болып табылады. Гамма-интерферон II типті интерферондардың жалғыз өкілі. Бұл цитокин туа біткен иммунитет ретінде табиғи киллер, табиғи Т киллер жасушаларымен және антигеннің айрықша иммунитетінің белсенуі кезінде Th1 CD4, CD8 лимфоциттерімен синтезделеді. Осы ақуыздың өндірісі зор практикалық маңызы бар.**

**Берілген жұмыста біз *E.coli* жасушаларында рекомбинантты адамның гамма-интерферонын алғанымызды баяндаймыз. Ген *de novo* ПТР әдісімен құрастырылып, нысана гені бар бірнеше**

**плазмидты векторлар алынды. Алынған генетикалық құрылымдарда нысан ген гекса-гистидинді белгімен және белгісіз клондалды. Рекомбинантты ақуыздың экспрессиясы үшін *Rosetta2 (DE3)* штаммы қожайын-жасуша ретінде қолданды. *E.coli* жасушаларында рекомбинантты ақуыздың жоғары дәрежедегі синтезіне индукция жағдайларын оңтайландыру арқылы қол жеткізілді. ИПТГ-ның оңтайлы концентрациясы 0,005 мМ тең. Рекомбинантты ақуыздың *E.coli* жасушаларында қосылыс денелер түрінде жинақталады. Рекомбинантты адам гамма-интерферонының жоғары тазалығына хаотропты агенттер көмегімен жүргізілген көп қадамды экстракция арқылы қол жеткізілді. Ақуыздың тазалануы Q және SP сефарозалары арқылы аниондық- катиондық хроматография көмегімен орындалды.**

**Түйін сөздер: Гамма-интерферон, цитокин, рекомбинантты ақуыз.**