



Complex Protein Expertise

The production and analytical assessment of proteins produced from *E. coli* can be challenging and complex. At WuXi Biologics, our expert team of highly trained scientists can perform a wide array of protein research activities and analyses from *in vivo* biotinylation, major histocompatibility complex (MHC) studies, cytokine production and screening and protein refolding. Our team has extensive experience with difficult proteins and routinely conducts projects requiring protein expression optimization, evaluation and use of a wide variety of labels, tags, and chaperones, and the fermentation and co-expression of proteins.

Experience with Difficult-to-Express Proteins

Intracellular Expression:

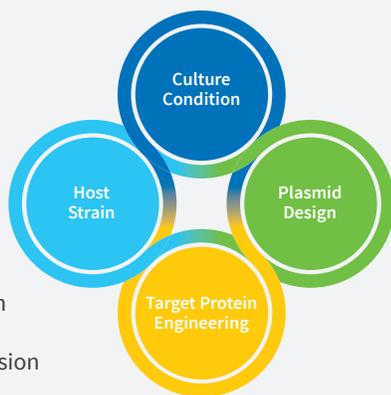
- Soluble
- Inclusion body

Secreted Expression:

- Periplasm

Different Strains (K/B) and Hosts Available:

- Protease deficiency
- Disulfide bond enhancer
- Minimal leaky expression
- Low-rate producer
- Low temperature expression
- Low endotoxin



Shake Flask Studies:

- 0.1-10 L various parameters evaluated:
Temperature, inducer, cell density, culture time, media, etc.

HTP Refolding Platform

Chaperones:

- HSP: DNaK, DNaJ, GrpE, GroEL/ES

Fusion Tag and Removal:

- His/Flag/GST
- MBP/NusA: solubility, folding
- SUMO/Ubiquitin: solubility

Signal Peptide:

- Sec/Tat pathway

Vector, Promoter, Copy Number, and more

E. coli Platform Features:

Projects start from customer-provided amino acid sequence (with codon optimization), DNA sequence, or plasmids

Use of multiple purification steps to deliver high quality product

A toolbox for hard-to-express targets, including host strains, culture conditions, and plasmid designs

High throughput screening for evaluation of protein refolding conditions (over 200 refolding conditions established)

Cell-free expression system available for the screening of hard-to-express proteins and various mutants

Protein expression condition optimization

Flexible product purification and analytical QC packages



E. coli Large-Scale Fermentation

Service Scope	Advantage
Plasmid Construction	Various Promoter Systems
Shake Flask Optimization	Different Feed Control: DO/pH-stat, Exponential
1-5 L Scale up	Higher Titer (1-20 g/L)
Up to 50 L	Short Culture Period (1-5 days)

Package Selection Guide

Our various *E. coli* protein production packages can start with an optional protein expression optimization study. We offer multiple scales of production up to 50 L, purification of both the soluble protein and inclusion body fractions and flexible QC analysis.

Popular packages	Features	Timeline	QC and Deliverable
Expression optimization	4-8 conditions	1 week	Expression report, soluble vs. insoluble fractions
Refolding screening	96 DWP, >200 conditions to choose	2-3 weeks	Refolding report
1 L (shake flasks)	Tag removal, biotinylation, endotoxin removal	3-4 weeks	Concentration, Caliper (R/NR) or SDS-PAGE, LC/MS
5 L, 10 L and 50 L (fermentation)	Fed batch	4 weeks	
Additional QC	RP-HPLC, bioburden, DSF, DSC, SPR, LC/MS, SEC-MALS, peptide mapping, cIEF, residual DNA, ELISA, Western blot, freeze thaw study, micro-developability testing		

Case Study: *E. coli* Expression Optimization

In this case study, we performed an expression optimization study to improve protein expression.

Key Challenge:
Low expression

Expression condition optimization

Expression Analysis

Final Products

Optimization

Expression analysis

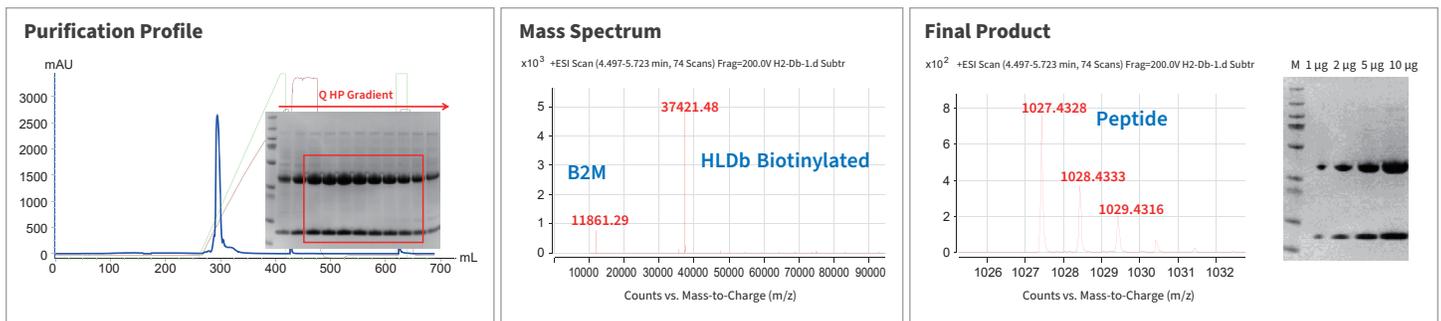
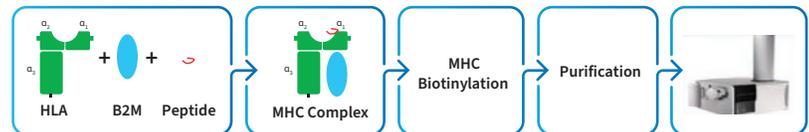
Final Products

Parameters	Original	Optimized
Medium	Platform Medium	Optimized Medium
Strain	BL21 (DE3)	BL21 star (DE3)
Temperature	20	37
Titer (mg/L)	1-5	>100
Time (h)	24	24

Case Study: MHC Production

Major histocompatibility complex (MHC) proteins are ternary complexes consisting of three components. They are notoriously hard to express and assemble, while retaining the proper peptide presenting function.

Over 260 MHC proteins produced from *E. coli* (0.1 L to 50 L)



About WuXi Biologics

WuXi Biologics is a leading contract research, development and manufacturing organization (CRDMO) that provides end-to-end capabilities to healthcare organizations worldwide. With operations in China, the United States, Ireland, Germany, and Singapore, we enable our partners to effectively and efficiently bring biologics and vaccines to patients worldwide through our comprehensive and high-quality drug development model.

The world's leading global single-source platform from concept to commercialization.

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Learn more:
Protein Sciences: Early-Stage R&D
E. coli Platform

