DIY Insulin: A Best Practices Manual for Safe and Effective Production

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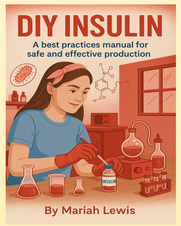
Overview

My manual for safe and effective insulin production will provide researched based steps for insulin production at home keeping in mind safety, legal considerations, ethical concerns and best practices. My manual will include information to guide practices through the following steps:

1. Bacterial Transformation & Plasmid Optimization
2. Fermentation & Scale-Up
3. Protein Expression & Folding
4. Protein Extraction & Isolation
5. Protein Purification
6. Insulin Folding & Cleavage
7. Quality Control & Bioactivity Testing
8. Legal & Ethical Considerations



For the full paper with all recommendations, scan here.

For the easy-to-follow guidelines, scan here.

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Step 1: Bacterial Transformation & Plasmid Optimization

Best bacterial strains for recombinant protein production in *E. coli*

BL21 is the Escherichia coli stain of choice. Primary due to strain BL21 being defective in cytoplasmic protease, Lon, and an outer layer membrane protease due to mutations. These mutations allow intracellular accumulation of heterologous proteins to happen at reasonable rates while minimizing the degradation of proteins during purification. (Wiley Online Library)

Comparison of heat shock vs electroporation in bacterial transformation

Heat Shock is more practical and cost effective method for small scale insulin production. If properly demonstrated, heat shock can yield a high number of transformation. Electroporation is more efficient for systems that require higher transformation efficiency, especially for eukaryotic cells. (Medilam)

Antibiotic selection in *E. coli* transformation for recombinant proteins

Kanamycin is the overall antibiotic of choice kanamycin is stable up to 24 hours, provides the selective pressure needed and is suitable for overnight cultures or multi-day fermentations. If you're working in non-sterile environments (common in DIY), random bacteria can enter. kanamycin kills any bacteria that isn't carrying the kanamycin-resistance gene. (Biosynth)

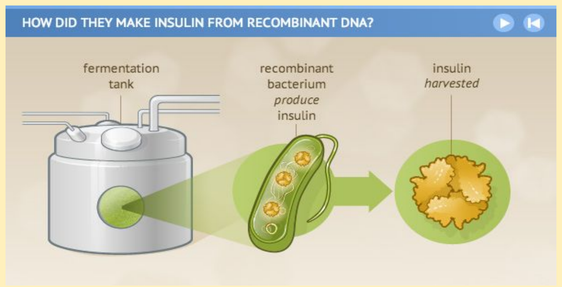
Step 2: Fermentation & Scale Up

Comparison of batch vs fed-batch fermentation for recombinant protein expression

Fed-batch fermentation generally has higher yields, less acetate production, an extended expression phase and growth is more controlled. It has a more complex setup, higher in cost, and needs optimization. Batch fermentation has a simple setup, low cost, ideal for beginners and is well suited for small scale production. You can start with Batch fermentation then scale to fed-batch once your system becomes optimized.(NIH)

Effect of oxygen levels on *E. coli* protein production

There are four types of oxygen levels, anaerobic (0%),microaerobic (~1-5%),Moderate (~20-30%) and High (~40-60%+). The target range for insulin production is Moderate (20-30%). With moderate oxygen levels full aerobic respiration can happen. ATP levels are high , stress levels are low and the plasmid is used efficiently. (NIH)



(NIH)

Step 3: Protein Expression & Folding

Effect of temperature on protein folding in *E. coli*

Temperatures between 25-37°C are optimal for harvesting Escherichia coli. At this range of temperatures Escherichia coli can effectively maintain tight coupling during translation and growth without the need to produce more ribosomes. Heat causes current ribosomes to translate faster. (ASM)

Optimization of IPTG concentration for recombinant protein expression

0.1–0.5 mM IPTG is the optimal level of IPTG as it expresses enough protein but also keeps cells healthy. At mid-log phase IPTG levels should be at (OD600 = 0.6–0.8) for healthy cells. If your protein becomes insoluble Try lowering IPTG and temperature. If cells have low expression Increase IPTG slightly (0.25–0.5 mM). Cells that show signs of stress or slowed production rate lower IPTG or delay induction. 0.05 is good for soluble expression, 0.1 helps protein folding and good for complex proteins, 0.5 is common starting level and 1.0 and above is very strong leading to stress and misfolding. (BMC)

Step 4: Protein Extraction & Isolation

.Endotoxin removal techniques for recombinant protein purification

Triton X-114 Phase Separation is an effective method to remove Endotoxins. Triton separates proteins by hydroplasty into two layers using heat.(25–37°C). Your top layer is hydrophilic while your lower layer is hydroponic. After centrifugation happens you collect the top layer containing your protein and discard the bottom layer the (endotoxins). You should repeat the process 2 to 3 times to maximize purity.(American Pharmaceutical)

Comparison of cell lysis methods for protein extraction in *E. coli*

Triton X-100 follows the same procedure as Freeze-thaw but includes lysozyme and triton x-100. Lysozyme breaks down the peptidoglycan and Triton X-100 is a mild detergent that helps dissolve cell membranes .Triton helps release proteins gently and Lysozyme by breaking the peptidoglycan causes the cell to burst open. Together the work to weaken the cell so proteins can be released. While freeze-thaw alone gives promising results, Freeze-thaw + lysozyme + Triton X-100 enhances the process with minimal price increase or complications. (ThermoFisher)

Step 5: Protein Purification

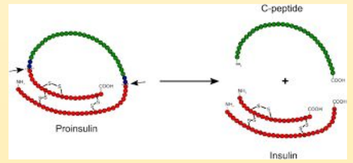
Comparison of affinity chromatography vs ion exchange for insulin purification

Affinity chromatography separates proteins by specific tags on insulin (His-tag). Affinity chromatography works by insulin sticking to a resin that binds to the his-tag. Purity levels are very high in the set up is easy, just add, wash and elute. Ion exchange chromatography separates proteins by the charge of insulin compared to other proteins charges. Ion exchange chromatography works by insulin sticking to a resin based on a positive or negative charge at certain pH levels. Affinity chromatography is ideal for DIY in insulin production. (NIH)

Step 6: Insulin Folding & Cleavage

Chemical vs enzymatic cleavage of proinsulin

Proinsulin has three parts, a, b, and c chain. To produce active insulin, the c chain must be removed. Enzymatic cleavage mimics the human pancreas, natural production of insulin.The 3 steps to this method are refolding pro insulin, adding enzymes to cut c-peptide and purifying final insulin. Chemical cleavage uses harsh chemicals to break proinsulin, specific chemical points, if not carefully controlled, can cause side reactions, and damage to the protein. (NIH)



(ResearchGate)

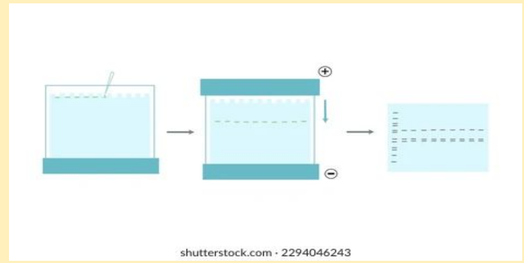
Step 7: Quality Control & Bioactivity Testing

SDS-PAGE vs mass spectrometry for insulin characterization

SDS-PAGE is an insulin characterization method that separates proteins based on their molecular weight. If a band is around 6 kDa its insulin, a singular clear band means your sample is pure, multiple bands are a sign of impurity and band shifts indicate chain cleavage.Use Mass spectrometry when producing clinical grade insulin, want to confirm exact structure, and have access to the lab core. (Wiley Online Library)

"Receptor binding assays for insulin bioactivity testing

Receptor binding arrays for insulin bioactivity testing is a lab test that checks if your insulin combines with the insulin receptor. If your insulin binds properly, it is active and folded correctly. Use this test to find out if your insulin works and functions as insulin should. To lower blood sugar insulin needs to attach to insulin receptors on cells if it can't bind, it's nonfunctional. (NIH)



(Shutterfly)