GENE AND CELL TECHNOLOGIES

E. *coli* Protein Extraction Kit

- Full Manual -

Summary

This kit provides the necessary reagents to lyse cultures of E. *coli* BL21 strains and extract recombinant proteins in their biologically active form. At the end of this procedure, the protein is obtained in a form ready for downstream purification methods, such as ammonium sulfate precipitation, ion-exchange chromatography, His_6-affinity chromatography and others. The small size kit provides sufficient reagents for 1 liter of culture, and the medium size for 4 liters.

Components and Storage Conditions

Lysozyme	Dissolve Lysozyme in Lysis Buffer; Then freeze at -20°C or colder. The solution must freeze.
DNAse I	Dissolve DNAse in DNAse Storage Buffer. Store at +4°C or -20°C. The solution must not freeze.

Calcium Chloride	Room temperature or colder.
DNAse Storage Buffer	Room temperature or colder.
Lysis Buffer	Room temperature or colder.

It is convenient to simply place the entire small tube tray in the -20° freezer.

Preparation

Before you begin, the enzymes need to be dissolved in their respective storage solutions:

(1) Dissolve Lysozyme in Lysis Buffer.

Use 1 ml of Lysis Buffer for the small kit, and 4 ml for the medium kit. This will achieve a concentration of 10 mg/ml Lysozyme. Swirl the vial around gently, until the Lysozyme is completely dissolved. This preparation must be stored frozen at -20°C or colder.

(2) Dissolve DNAse I in DNAse Storage Buffer.

Add the entire vial of DNAse Storage Buffer to the DNAse vial. This will achieve a concentration of 1 mg/ml DNAse. Swirl the vial around gently, until the DNAse is completely dissolved. This preparation must be stored in liquid form at +4°C or -20°C.

Now you are ready to proceed with the lysis protocol on page 4.

Background: Storage Conditions for the Enzymes

The enzymes provided with the kit, Lysozyme and DNAse I, have individual storage requirements as outlined in the table on page 1. Although many other proteins "forgive" and survive deviations from their ideal conditions, Lysozyme and DNAse are very particular about that – different conditions will reliably destroy these enzymes and render them unfit for use with the kit.

Both enzymes are provided as lyophilized powder. In this form, they are stable at room temperature near-indefinitely (>1 year tested at Gene And Cell Technologies). It is only once they are re-suspended in liquid form that they become vulnerable to storage-stress.

Lysozyme is a small (14 kDa) protein, which is prone to aggregate and fall out of solution when stored in liquid form. As little as overnight incubation in the fridge can cause it to precipitate and become completely inactive. Lysozyme must therefore be stored frozen. When frozen, individual lysozyme molecules are immobilized and cannot reach each other to aggregate. Due to its small size, Lysozyme can spontaneously refold after minor damage, and is able to regain its full activity upon thawing. At Gene And Cell Technologies, we have tested >30 freeze-thaw cycles of Lysozyme dissolved in Lysis Buffer, and found complete preservation of its activity throughout. Lysozyme in Lysis buffer will freeze at -20^{o}C. Some older freezers can be significantly warmer. It's best to double-check and make sure that the solution does indeed freeze. Ice formation should be readily visible by eye.

DNAse I (31 kDa) behaves just the opposite of Lysozyme. The DNAse will be inactivated completely by as few as 5 freeze-thaw cycles and does not regain its activity. One cycle is permissible, for example if it becomes necessary to store the enzyme for the very long term at -80^{o}C, or if single-use aliquots are made. However, repeated freeze-thaw cycles must be avoided. The DNAse is stable when stored as a cold liquid, at either +4^{o}C (fridge) or -20^{o}C (regular freezer). A cryo-protected DNAse storage buffer is provided, which will prevent freezing of the DNAse at -20^{o}C when resuspended as instructed. Therefore, please make sure that your freezer is not set significantly colder than -20^{o}C.

Following these guidelines will reliably preserve the enzymes for very long periods (> 1 year tested at Gene And Cell Technologies). If the enzymes get denatured by accident, the most effective solution is to ask us for replacements by using the contact form at www.geneandcell.com/pages/contact.

Background: Recombinant protein expression in E. *coli*

Escherichia *coli* provides a basic, low-barrier-of-entry system to manufacture recombinant proteins. E. *coli* grows easily in inexpensive media, such as LB-broth. Protein-expression strains are easy to obtain, and many are not restricted by any form of intellectual property. The genetic circuits for protein expression in E. *coli* are comparatively simple to understand and design. Vectors directing high-level expression in E. *coli* are widely available. In the age of commercial gene synthesis, anyone can easily obtain their favorite gene, thoroughly optimized for E. *coli* expression, for a few hundred dollars.

Especially for proteins of bacterial or phage origin, E. *coli* can often produce high yields of native, biologically active protein. However, for proteins from other kingdoms, such as human, plant or archaeal proteins, misfolding and insolubility are common problems. Non-bacterial recombinant proteins often aggregate in the form of insoluble inclusion bodies in E. *coli*. In some cases, aggregation problems can be managed through techniques like fusion proteins, codon choice modifications, reduced-temperature expression, chaperone co-expression or chemical refolding. But these techniques do not necessarily lead to an acceptable level of success in all cases. It is important to be aware of these limitations, before choosing an expression system.

Generally, E. *coli*'s ability to secrete recombinant proteins into the medium is limited. Most proteins are therefore produced in the cytoplasm. This necessitates a method to lyse E. *coli*, in order to obtain an extract containing the protein of interest. The the purpose of this kit is to lyse E. *coli* cells, while protecting recombinant proteins and preserving their native state and biological activity. The kit is inexpensive to use on an ongoing basis, does not require any expensive specialized equipment and can be scaled up for commercial production.

E. *coli* comes in many different strains, which have been genetically optimized for a variety of purposes. The most commonly used strains are based on "10-beta" for nucleic acid production, and on "BL21" for protein production. This kit can be used with BL21 and its derivative strains.

For protein expression, a wide variety of commercial and academic systems are available. Due to the diversity of systems, we do not provide specific instructions for this step. The manufacturer of each system would be the best source for detailed instructions. In the typical expression system, E. *coli* BL21 would be transformed with a plasmid containing the gene for the protein of interest under a promoter that is inducible by the lactose analog IPTG. The culture is grown under antibiotic selection to about half its maximal optical density. Then IPTG is added to a concentration of 1-4 mM. Protein production will proceed for a period between 1-12 hours. The optimal timing depends on the protein of interest, and will vary. Protein production should be assessed over time with a suitable analytic method, such as SDS-PAGE. Once protein production peaks, the live culture containing the protein of interest is your starting material for the lysis protocol below.

Lysis Protocol

Make sure you have prepared the enzyme solutions as directed on page 1 “preparation”.

(1) Pellet an E. *coli* culture containing your protein of interest by centrifugation.
4000 x g at 10 minutes should be sufficient. Refrigeration is helpful for some proteins.

(2) Pour the supernatant off and remove residual liquid.
You can remove the residual liquid from the tube walls by doing a second, brief spin step. Alternatively, invert the centrifuge tube on a paper towel for a few minutes.

(3) Resuspend the pellet Lysis Buffer at 1/10th of the original culture volume.
For example, use 100 µl of Lysis Buffer if the original culture volume was 1 ml. Make sure the pellet is completely dispersed and no clumps remain. It is convenient to use a pipet tip on small scale, or an electrical cocktail stirrer or dough mixer on large scale.

(4) Add 1/1000th of the original culture volume of Lysozyme.
For example, add 1 µl of Lysozyme, if the original culture volume was 1 ml. Mix well.

(5) Freeze the suspension at -20^{o}C.
At this step, ice-crystal formation will cause minor cracks in the cell wall. This will later allow the lysozyme to penetrate into the cell wall and digest it completely. Make sure the cell suspension freezes completely. Overnight freezing at this step will be sufficient at almost any scale, and may be convenient in terms of work-flow.

(6) Thaw the cell suspension to 37^{o}C in a water bath.
When it is fully warmed up, Lysozyme becomes active. If your protein is soluble, the solution should markedly clear up at this step. If the protein is insoluble, then inclusion bodies may appear so milky that the clearing of the cell walls is not visible at all.

(7) Add 1/1000th of the original volume of each $CaCl_2$ and DNAse. Incubate for 15 min.
Together with its co-factor Ca^{2+}, the DNAse will digest long genomic DNA strands released from the cells. This reduces the viscosity of the lysate, permitting downstream steps such as column purification. Invert or mix the vial repeatedly, to make sure DNAse permeates the entire solution, as the viscosity is gradually reduced. The viscosity can be monitored by dripping the lysate through a standard 200 µl pipet tip. When it drips freely like water, without drawing stings, then the DNA is sufficiently broken down.

Your lysate containing your recombinant protein is ready for downstream processing.