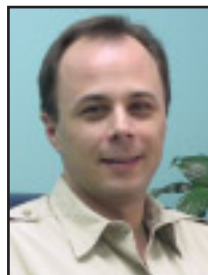


# A Rapid Method to Assess Protein Overproduction and Solubility in *E. coli* Using EasyLyse™ Bacterial Protein Extraction Solution

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degradation of messenger RNA. In *E. coli*, RhlB and PPK are components of a multi-enzyme RNA degrading machine called "the degradosome." The functional consequences of assembling enzymes into this complex are being investigated by measuring the enzymatic properties of purified degradosomes and their component enzymes. Medical College of Georgia, 1120 15th Street, Augusta, GA 30912 Tel (706) 721-7659, Fax (706) 721-6608, E-mail cburns@mcg.edu

## Introduction

Overproduction in *E. coli* is a powerful method that simplifies homogeneous purification of recombinant proteins for structural and functional studies. Typically achieved using the method originally developed by Studier<sup>1</sup>, the gene of interest is cloned under control of a phage RNA polymerase promoter to enable hyper-transcription of the messenger RNA. Not all genes respond to hyper-transcription; some readily produce abundant protein, while others do not.

One potential problem of protein overproduction in *E. coli* is the tendency of some proteins to form inclusion bodies, insoluble aggregates of the protein. This phenomenon is even observed for certain native *E. coli* proteins when overproduced in their normal environment. If insolubility of overproduced proteins is overlooked, much time and effort can be wasted trying to purify a protein from solution, when most of the desired protein was actually discarded with the cellular debris.

A rapid method to assess the solubility of an overproduced protein uses EPICENTRE's EasyLyse™ Bacterial Protein Extraction Solution. With this method, a single experiment provides two levels of information about the protein of interest -

the extent of overproduction and the solubility of the overproduced protein. The EasyLyse method (outlined in Figure 1) is straightforward and applicable to both pilot scale experiments and to prescreening large-volume overproduction cultures.

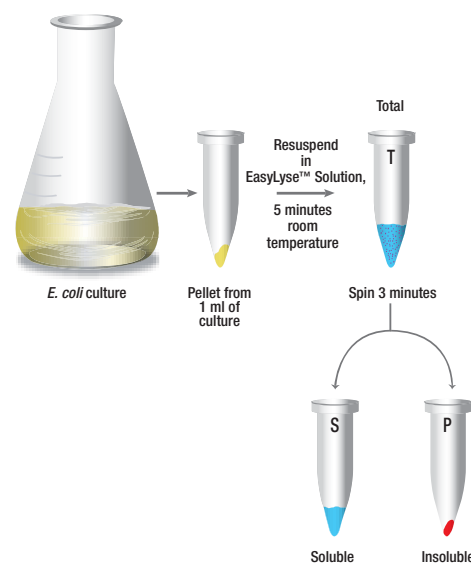
## Methods

From control ("empty vector" or pre-induction) and induced cultures, 1-ml cell samples are pelleted and resuspended in 200  $\mu$ l of EasyLyse Solution. After a 5-minute incubation at room temperature, a 100- $\mu$ l sample, representing the total protein, is removed and the remainder of the sample is centrifuged at full speed for 3 minutes, separating insoluble proteins from the soluble proteins, which remain in the supernatant. The supernatant is transferred to a new tube and the entire pellet fraction is solubilized in 100  $\mu$ l 1X SDS loading buffer. To assay the samples, 15  $\mu$ l of the total and supernatant fractions are each mixed with 5  $\mu$ l of 4X SDS loading buffer. The samples and 15  $\mu$ l of the solubilized pellet fraction are heated at 95°C for 1 minute and separated by SDS-PAGE. These volumes are appropriate for analysis of mid-log phase cultures on mini-gels with Coomassie® blue staining. Collecting duplicate cell samples is recommended.

## Results and Discussion

The usefulness of testing solubility with EasyLyse Solution is illustrated in Figure 2. Panel A shows the results obtained using a common method to assess the extent of protein overproduction (heating cells in SDS). Two *E. coli* proteins, RNA helicase B (RhlB) and polyphosphate kinase (PPK), appear to be highly overproduced compared to the control. However, because a denaturing detergent is used in this procedure, soluble and insoluble proteins are indistinguishable. Without further information, these results suggest that both proteins could be purified from solution using standard strategies. This is not the case.

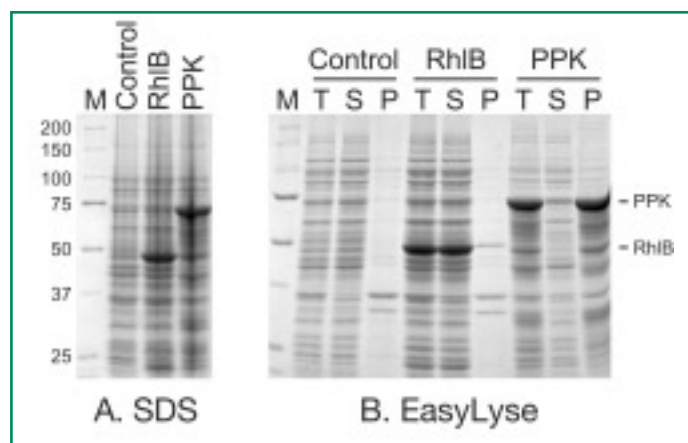
Panel B shows analysis of the same cultures using the EasyLyse method. As in the SDS lysis approach, examination of the



**Figure 1: Diagram of overproduction and solubility testing procedure using EasyLyse™ Bacterial Protein Extraction Solution.** A 1-ml aliquot of an overnight bacterial culture is centrifuged to pellet the cells, the supernatant is removed and the cell pellet is suspended in 200  $\mu$ l EasyLyse Solution. After 5 minutes at room temperature, 100  $\mu$ l is removed from the tube (TOTAL protein fraction) and the remaining lysed cells are centrifuged at full speed for 3 minutes. The Supernatant (soluble protein fraction) is removed to a clean tube and the Pellet (insoluble protein fraction) is solubilized in 100  $\mu$ l 1X SDS loading buffer.

TOTAL fractions reveals that both RhlB and PPK are overproduced relative to the control. However, comparison of the SUPERNATANT and PELLET fractions shows that the majority of overproduced RhlB is soluble, while overproduced PPK is not. The EasyLyse experiment indicates that standard strategies are appropriate for RhlB purification, but would be fruitless for PPK purification. This information could save substantial effort by informing the researcher early on that alternative strategies must be employed to either improve PPK solubility *in vivo*, or to purify insoluble PPK with subsequent *in vitro* solubilization and refolding.

The EasyLyse method takes about 15 minutes to prepare samples from two or three cultures for SDS-PAGE analysis. EasyLyse Solution is particularly well suited to protein purification experiments because it is



simple to use and reported to lyse cells more completely and preserve enzyme activity better than other chemical lysis methods.<sup>2</sup> Methods that do not employ denaturing detergents, such as physical disruption, can be used to assess protein solubility, but these generally require specialized equipment or expertise and may therefore be less attractive.

Before introduction of the EasyLyse Solution, a similar method using EPICENTRE's ReadyPreps™ Protein Preparation Kit was used to study factors influencing overproduced PPK solubility. Because the procedures are robust and do not require significant expertise, this project proved an excellent training opportunity for two student researchers. Without any significant prior research experience, they determined the impact of cell culture conditions and

of chaperone co-overproduction<sup>3</sup> on PPK solubility. EasyLyse Bacterial Protein Extraction Solution now offers a simple, cost-effective approach to monitoring protein overproduction and solubility.

#### Acknowledgments

Jiaqi Li is thanked for his assistance with the experiments. Amelia Bozeman and Frantz Duchatellier, Research Apprentices sponsored by the MCG Office of Special Academic Programs, used the ReadyPreps method to study PPK solubility.

#### References

1. Studier, F.W. and Moffatt, B.A. (1986) *J. Mol. Biol.* **189**,113.
2. Jarvis, B.W. (2003) *EPICENTRE Forum* **10**(3), 4.
3. Castanie, M.P. et al. (1997) *Anal. Biochem.* **254**, 150.

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