A Time Course Induction Study of Recombinant Protein Expression in *E. coli*

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INTRODUCTION

Recombinant protein production in the form of *E. coli* inclusion bodies is a complex process that depends on a number of parameters. These parameters can be optimized individually, or in relation with each other, using multi-variate techniques to obtain the highest yield-to-production cost ratio.

Optimization studies can be time-consuming since they often involve labor-intensive techniques. For inclusion body proteins, the process starts with disrupting intact cells to release their inclusion bodies. The recombinant protein is then released by solubilizing the inclusion bodies, and the desired product is further purified to make its quantification meaningful.

In this application note, the ProteoSpin™ Inclusion Body Isolation Micro Kit was used in a simulation study for optimizing the parameter for duration of induction time during the production of a recombinant protein. Starting with a fresh culture of an inclusion body-producing bacterial clone, cells were induced in a fermentation batch for protein synthesis. During incubation, samples were obtained at defined time points and their recombinant protein was isolated. The relative amounts of protein made were then displayed and compared using SDS-PAGE. This study demonstrates that the ProteoSpin™ Inclusion Body Isolation Micro Kit is an effective tool in the time course analysis of gene expression.

METHODS AND MATERIALS

The materials and methods used to evaluate the ProteoSpin™ Inclusion Body Isolation Micro Kit (P/N 10300) have been separated into discrete phases:

- Bacterial Growth
- Isolation of Inclusion Bodies
- ProteoSpin Isolation of Inclusion Body Proteins
- SDS-PAGE Analysis of Inclusion Body Proteins

Bacterial Growth

A stock culture of *E. coli* expressing a 40 kDa protein was streaked on selective agar plates containing antibiotics and incubated for 16 hours at 37°C. One colony was picked using a sterile wooden stick and inoculated in a test tube with 2 mL of LB (Luria broth) that contained antibiotics. The tube was placed in a 37°C shaking waterbath and incubated for eight hours. The culture was then transferred to a 2 mL microcentrifuge tube and centrifuged for one minute at 14,000 x *g*. A 2 mL volume of fresh LB with antibiotics was used to resuspend the cell pellet. This was then added to 50 mL of LB with antibiotics and placed in a 37°C shaking waterbath until the OD_{600} reached approximately 0.6. At this point, a 1.5 mL aliquot of culture was removed to a fresh microcentrifuge tube and stored at 4°C (negative control). To the remainder of the culture, 200 µL of 100 mM IPTG was added and the flask was incubated for another three hours in the 37°C shaking waterbath. During this time, 1.5 mL aliquots were removed to fresh microcentrifuge tubes every twenty minutes. The tubes were centrifuged for one minute at 14,000 x *g* and the media was removed. The cell pellets were stored at -70°C until processed.

Isolation of Inclusion Bodies

After removing the final aliquot at the three-hour point, the Inclusion Body Isolation Micro Kit was used to complete the isolation. Two hundred microliters of cell lysis reagent was added to the thawed cell pellets, and inclusion body isolation was carried out as described in the Application Manual. Once the final inclusion body pellets were obtained, 50 µL of solubilization reagent was added to each tube. Pellets were dissolved by vigorous vortexing and the volume was adjusted to 100 µL with the addition of sterile deionized water.

ProteoSpin Isolation of Inclusion Body Proteins

The theoretical p*I* of the 40 kDa protein is 5.56 as determined from the ExPASy Molecular Biology Server (www.expasy.org). With this information, it was determined

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that the acidic protocol was appropriate. Fifty-microliter volumes of dissolved inclusion bodies were removed to fresh tubes and 200 µL of sterile deionized water was added to each. Acidic pH binding buffer was added and the tubes were vortexed to mix. ProteoSpin™ columns were activated twice and the pH adjusted protein solution was subsequently processed. The flowthrough was discarded and the column was washed twice using the column activation and acidic wash buffer. Following the final wash, the columns were transferred to fresh microcentrifuge tubes containing 5 µL of neutralizer solution. Proteins were eluted twice using 25 µL of elution buffer into a single microcentrifuge tube.

SDS-PAGE Analysis of Inclusion Body Proteins

A 12.5% polyacrylamide gel was prepared for SDS-PAGE analysis. A 5 µL aliquot of each elution was transferred to a fresh microcentrifuge tube, to which 3 µL of 3X loading dye had been added. The samples were mixed, boiled for two minutes, and then loaded onto the polyacrylamide gel. The gel was run for 45 minutes at 200 V/6.5 cm and stained using Coomassie Blue R-250. The resulting gel was scanned for subsequent visual analysis.

RESULTS AND DISCUSSION

Visual examination of the gel in Figure 1 reveals that over a period of three hours, the amount of the recombinant protein (appearing as a single band on SDS-PAGE) increases from background levels to an apparent maximum. The full-length protein started to appear between 40 and 60 minutes post induction, and continued to increase until a maximum level was reached after 160 minutes. From this information it can be inferred that a minimum incubation time of three hours after induction will produce a substantial amount of protein. Longer incubation may not result in any significant increase in yield, a speculation that can be further tested in subsequent experiments.

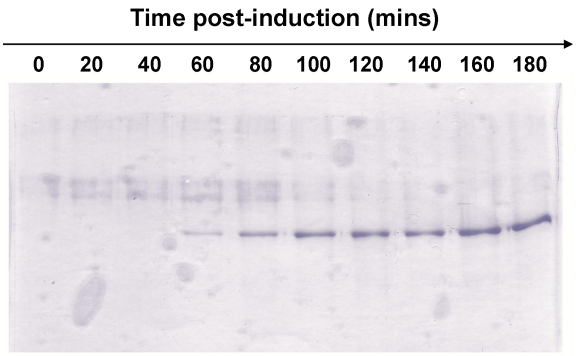

Figure 1: Time course study for the expression of a 40 kDa protein. Expression was induced with 0.4 mM IPTG and incubated at 37°C. Aliquots were removed at indicated intervals and purified using the ProteoSpin™ Inclusion Body Isolation Micro Kit. Proteins made visible by Coomassie Blue staining.

CONCLUSION

The ProteoSpin™ Inclusion Body Isolation Micro Kit is useful in optimizing a parameter important in inclusion body production of a recombinant protein. A simple time course experiment was carried out to determine the minimum incubation time after induction needed to attain maximum expression levels. The rapid purification method enables processing of 10 samples, from induced culture to SDS-PAGE analysis, in a period of one hour. This simulation study demonstrates that the ProteoSpin ™ Inclusion Body Isolation Micro Kit simplifies many of the steps in purifying recombinant proteins, and in doing so proves to be a useful tool in parameter optimization.

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