

Optimized Protein Expression Growth in 96-Well Plates

HiGro Application Note #314

ABSTRACT

The growing emphasis on proteome studies has propelled protein research into the highthroughput market. The emergence of large scale, proteomic screening tools has created an increased demand for protein libraries. Furthermore, researchers in structural and functional genomics are under pressure to streamline the characterization of target proteins. These applications require tools that are high-throughput yet flexible enough to allow researchers to test a variety of expression conditions. This Application Note addresses some of these issues for an *E.coli* expression system using the HiGro® micro-cultivation shaker which provides a unique combination of small-orbital shaking, temperature and gas flow controls, and high microwell plate capacity. These features allow for optimization of protein expression growth in a 96-well shallow-well plate format to yield sufficient protein product for downstream applications. This Application Note describes a baseline protocol from which customers can refine parameters to suit the growth and expression requirements of their specific cell strains and plasmid types.

METHODS

Expression Strains

Protein expression growth experiments were conducted using two *E. coli* strains containing different GST fusion protein constructs. The first, pKG58 GXCDC33, contains the IPTG-induced *tac* promoter attached to the *S. cerevisiae* CDC33 ORF. In the second construct, pKG59 GXLEU2, the same promoter is attached to the LEU2 ORF.

Culture Preparation

Cultures were grown in the HiGro incubating shaker with an 8 mm shaking orbital and a New Brunswick® C24 incubating floor shaker with a ~25 mm shaking orbital. In the HiGro shaker, 0.3 mL of growth media (1x Terrific Broth with 100 µg/mL of ampicillin) were added to each well of a shallow-well, flat-bottom, polystyrene 96-well plate. Odd numbered columns in the plates were inoculated with 3 µl per well of seed culture. In the floor shaker, cultures were grown in 15 mL polypropylene snap-cap tubes containing 3 mL of the growth media. Each tube was inoculated with 30 µl of the same seed culture used for the HiGro shaker cultures. Two tubes were prepared for each strain. All cultures were inoculated immediately before being loaded into the shakers.

HiGro Shaker Growth Conditions

Approximately 30 minutes before use, the HiGro shaker was powered on and the temperature was set to 37°C for each of the four chambers. Chambers not used for the experiment were left at room temperature with the gas flow inlet valves kept closed. Air from a compressed, zero grade, air tank was used to supply gas flow to the HiGro shaker for all experiments. On the flowmeter, the gas flow rate to each chamber in use for this experiment was adjusted to approximately 5 SLPM (standard liters per minute) per chamber. (For example, if two chambers were used, the flowmeter was set to 10 SLPM.) The pulse timer was set to 0.5 seconds ON and 0.5 minutes OFF.

No gas flow delay was used. Immediately before loading the plates, the gas flow inlet valves were opened for the chambers that would be used.

Plates were loaded into the Digilab Microwell-Plate Cassettes without any lids or covers. If fewer than 8 growth plates were being loaded into a chamber, a paper towel wetted with distilled water was placed (flat) at the base of that chamber to maintain ambient humidity. After loading the cassette(s) containing the growth plates into the chamber(s), a clean, dry paper towel was placed over the top of each cassette regardless of the number of plates. The unused chambers were balanced with cassettes and water-filled balancing plates. (For convenience, prepare some balance plates or blocks that are filled with water and sealed with aluminum foil tape. These items may be left next to the HiGro shaker and used whenever balancing is necessary.) The shaking speed was set to 520 revolutions per minute (rpm). The cultures were incubated for 4 hours under these conditions.

Floor Shaker Growth Conditions

The floor shaker was powered on and the temperature set to 37°C approximately 30 minutes prior to loading the samples. For the cultures in 15 mL tubes, the caps were attached but not sealed tightly and the tubes were placed into holders in the floor shaker. For 4 hours the cultures were incubated at 37°C, shaking at 250 rpm.

Induction

After the 4 hour growth period, some of the cultures were treated with isopropyl β -D-thiogalactopyranoside (IPTG), an inducer of the lac operon, at the indicated concentrations (either a final concentration of 1 mM or 0.1 mM) and allowed to incubate with shaking at 30°C for an additional 16-18 hours. The shaking speed was reduced for cultures in the HiGro shaker to 400 rpm. All other conditions, as described in the HiGro and Floor Shaker Growth Conditions sections, remained the same.

Optical density (OD) readings of the cultures were taken after the induction period, within 30 minutes of removing the samples from the incubators with a Molecular Devices® SPECTRAMax® 190 microplate reader at a wavelength of 600 nm.

Total Protein Preparation

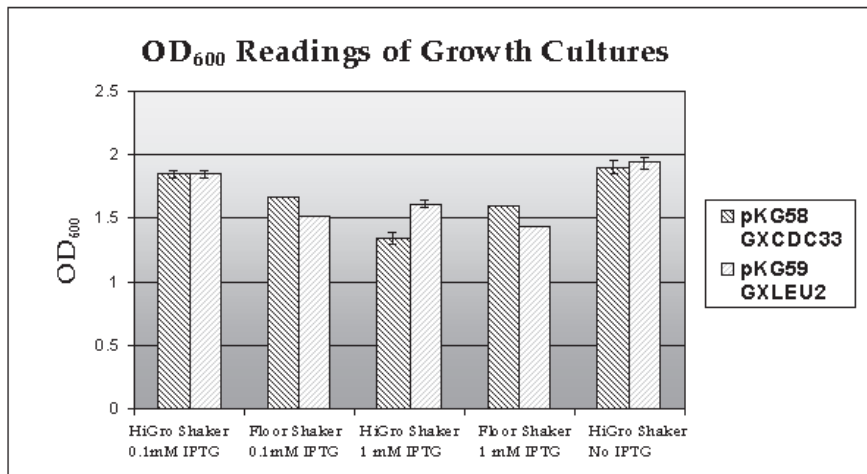
For total protein analysis, 0.3 mL of culture were transferred to 1.5 mL microfuge tubes. The cells were harvested by centrifugation at 14,000 rpm for 5 minutes. The supernatant was discarded and 0.5 mL of 2X Laemli buffer (60 mM Tris-Cl, pH 6.8, 2% SDS, 10% glycerol, 5% β -mercaptoethanol, 0.025% Bromphenol Blue) were added. The samples were then resuspended by pipetting. Samples were heated to 95°C for 5 minutes, then 15 μ l were loaded onto a 12.5% SDS-polyacrylamide gel (SDS-PAGE). Each gel also was loaded with one lane containing broad range molecular weight markers (Sigma- M 4038) and run until the dye front reached the bottom of the gel.

After the gels were run to completion, they were incubated for about 1 hour in a staining solution (0.1% (w/v) brilliant blue G (Coomassie), 25% (v/v) methanol, 5% (v/v) acetic acid) to visualize proteins. The gel was destained in 50% (v/v) methanol, 10% (v/v) acetic acid until background was reduced to the desired level. Typically this level was achieved with two, 2 hour incubations with one change of destaining solution. The gels were then hydrated and allowed to dry between cellophane.

RESULTS AND CONCLUSIONS

Comparisons of OD₆₀₀ readings of the cultures after the 18-hour growth period (Figure 1) show a reduction in cell yield from samples to which IPTG has been added. A direct comparison between reductions in the HiGro shaker and floor shaker cannot be made due to the lack of an un-induced floor shaker sample. However, the comparison data do indicate that the IPTG concentration has a significant effect on cell density in samples grown in 96-well plates in the HiGro shaker. Floor shaker samples show a minimal reduction in density due to the ten-fold increase in IPTG concentration.

Figure 1. Growth density comparison for samples grown in the HiGro shaker vs. a standard floor shaker.



Results from the isolation of total protein from the samples (shown in Figure 2) indicate that the decrease in IPTG concentration leads to a significant increase in protein yield for cultures grown in the HiGro shaker. For the floor shaker samples, a slight increase in target protein yield for pKG58 GXDC33 due to IPTG decrease is apparent in Figure 2a. However, the overall effect is less prominent than in the HiGro shaker samples. The data correlate with the optical density results shown in Figure 1. Based on these results, researchers may want to consider using a lower inducer concentration for protein expression in 96-well plates to balance potential cell yield losses.

Figure 2 also indicates that protein expression growth in 96-well plates can achieve sufficient protein product for downstream applications. By altering only one parameter, we have been able to achieve comparable results to a larger volume growth format. Furthermore, the HiGro shaker conditions for this baseline protocol were adapted with few changes from those conditions used for standard plasmid amplification in an E. coli host. With minimal effort, we have been able to determine a working protocol for protein expression in 96-well plates. These results demonstrate the HiGro shaker is the key to efficiently transitioning protein expression growth to a high-throughput 96-well plate format.

Genomics

Colony Picking

Cell Growth

DNA

Figure 2. Total protein isolation of samples grown in the HiGro shaker and a standard floor shaker.

Figure 2a

Microarraying

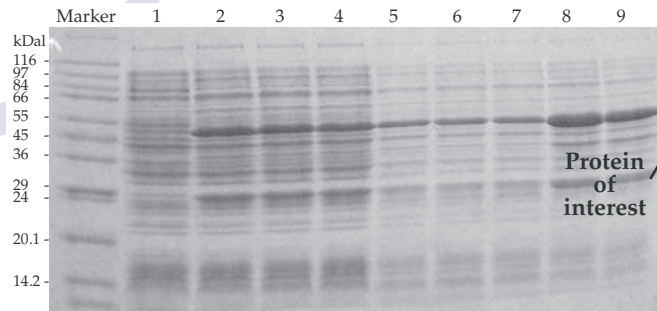
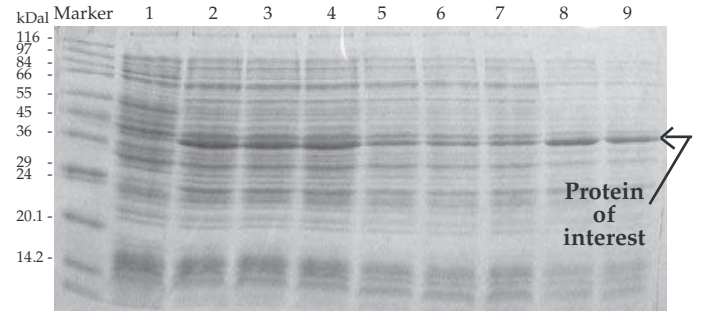


Figure 2b



Conditions for Figures 2a and 2b.

- Lane 1: Sample grown in HiGro shaker (96-well plate). Not induced.
- Lanes 2-4: Samples grown in HiGro shaker (96-well plate). Induced with 0.1 mM IPTG.
- Lanes 5-7: Samples grown in HiGro shaker (96-well plate). Induced with 1 mM IPTG.
- Lane 8: Sample grown in floor shaker (15 mL snap cap tube). Induced with 0.1 mM IPTG.
- Lane 9: Sample grown in floor shaker (15 mL snap cap tube). Induced with 1 mM IPTG.

Author: Sara Polgar

©2005 Digilab, Inc. This Application Note is an example and guideline and is not a guarantee of performance. Please contact Digilab with any questions regarding machine performance or potential protocols; we are happy to assist our customers in optimizing their protocols and machine performance. In addition, Digilab welcomes additional data and/or comments.

Worldwide Headquarters

Digilab, Inc.
 84 October Hill Road
 Holliston, MA 01746
 USA

Phone: (508) 893-3130
 Toll Free: (800) 935-8007
 Fax: (508) 893-8011
 E-Mail: info@digilabglobal.com

