

# Optimized E-Coli Growth in 96-and 384-Well Plates

## HiGro™ Incubated Shaker

### ▶ Abstract

Customers rely on the HiGro™ incubated shaker to provide high-throughput and reproducible results. The most common application that the HiGro shaker is used for is E.coli growth in 96- and 384-well plates to produce high-copy plasmid DNA. This Application Note provides protocol information for E.coli growth as well as tips on how to best take advantage of the unique controls and conveniences that the HiGro shaker offers. This information is intended to be baseline protocols from which customers can refine parameters to suit the growth requirements of their specific cell strains and plasmid types.

### ▶ Materials and Methods

#### 96-Well Growth

All 96-well growth experiments were conducted using E. coli strain DH10B carrying pUC19. Growth media was 1x Terrific Broth with 100 mg/μl Ampicillin. To allow for meaningful well-to-well and plate-to-plate comparisons of results, all growth plates were inoculated from the same glycerol stock using 5 μl per well. Two plate types were used in experiments; a clear, polystyrene, 96-shallow-well, flat-bottom plate and a Beckman® polypropylene deep-well, square-well, 96-well blocks. 0.3 mL/well and 1.0 mL/well of growth media were added to the plates and blocks respectively. Plates were inoculated immediately before being loaded into the incubators.

For comparison, cultures were grown in the HiGro shaker with an 8 mm shaking orbital and a New Brunswick® C24 incubating floor shaker with a ~25 mm shaking orbital.

Optical density (OD) readings were taken during growth experiments using a Molecular Devices® SPECTRAMax 190 microplate reader at a wavelength of 600 nm. All readings were taken in clear, polystyrene 96-shallow-well, flat-bottom plates. Prior to the reading of each plate, the microplate reader was “blanked” with a plate containing 0.1 mL/well of media. With the exception of the 0 hour time point readings, all cultures were diluted 1:10 into 0.1 mL of media for taking OD readings. For the OD readings, aliquots were removed from the bottoms of well-mixed plates.

#### **HiGro Shaker Conditions**

Approximately 30 minutes before use, the HiGro shaker was powered on and the temperature was set to 37°C for each chamber. 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 was adjusted to 6 SLPM (standard liters per minute) per chamber. (For example, if two chambers were used, the flowmeter was set to 12 SLPM.) The pulse timer was set to 2 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 cassettes without lids or covers of any kind. 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. Cassettes were loaded into the unused chambers and the chambers were balanced (if necessary) with water plates. The shaking speed was set to 550 revolutions per minute (rpm).

### **Floor Shaker Conditions**

The floor shaker was powered on and the temperature set to 37°C approximately 30 minutes prior to loading the samples. A piece of aluminum foil was placed loosely over each plate. A small hole was made in the foil above each well. The plates were then placed into the floor shaker and taped down. To maintain ambient humidity, a paper towel wetted with distilled water was also placed into the floor shaker. The floor shaker was set to 250 rpm.

### **384-Well Growth**

All 384-well experiments were conducted using *E. coli* strain Top10F' carrying pBluescript with an insert (3 kb total size). Cultures were grown in 384-shallow-well, flat-bottom plates with rounded-square wells. 90 µl of Magnificent Broth (MacConnell Research) containing 100 µg/ml Carbapenem was used per well. Growth plates were inoculated from the same overnight growth plate using 1 µl per well.

HiGro shaker conditions were the same described for 96-well growth with the following exceptions. 384-well growth experiments were conducted in a HiGro shaker with a 4 mm shaking orbital. The air flow rate was set to 1.5 SLPM per chamber and the pulse timer was set to 0.5 seconds ON and 0.5 minutes OFF.

At multiple time points over a 24-hour growth period, sample cultures were removed from the growth plates. Plasmid DNA isolations were conducted on each sample by pipetting 90 µl of the culture (total well volume) into a 1.5 mL tube containing 100 µl of alkaline lysis reagents. After 5 minutes at room temperature, neutralization reagents were added (100 µl). The sample was mixed and then pelleted. The supernatant was pipetted onto a commercially available DNA purification column. After completing washing steps, the sample was eluted into 50 µl of buffer. Isolation and purification steps were carried out at the removal time point. Optical density readings were not collected for 384-well growth experiments shown here.

## **▶ Results**

### **96-Well Growth**

A preliminary comparison of both deep- and shallow-well growth in the HiGro shaker and floor shaker indicates that for either format, the HiGro shaker facilitates significantly greater growth densities after an 18-hour growth period (Figure 1).

Time course comparisons of the HiGro shaker shallow-well growth and floor shaker deep-well growth show that the early rate of increase in the readings and the overall OD values are greater for the HiGro shaker than those of the floor shaker (Figure 2). These results indicate that cultures grew at an accelerated rate and to a higher cell density in the HiGro shaker.

Visual inspections of the cultures immediately after removal from the shakers at the 18-hour time point also indicate differences in distribution of cell matter in the plate wells of the HiGro shaker versus the floor shaker cultures (Figure 3). When viewing the cultures from the tops of the plates, a greater degree of turbidity, or cloudiness, is evident in the HiGro shaker cultures as compared with the floor shaker cultures. In shallow-well plates settled material is visible at the bottom of the floor shaker cultures, whereas the material is uniformly distributed throughout the wells in the HiGro cultures. These observations indicate that the floor shaker cultures are not experiencing vertical mixing and have “settled-out”, whereas material in the HiGro shaker plates is being mixed throughout the well. Furthermore, well-to-well cross-contamination in the shallow-well plate grown in the floor shaker was evident. No cross-contamination occurred for samples grown in either plate-type in the HiGro shaker.

Figure 1. 96-well shallow- and deep-well growth comparisons.\*

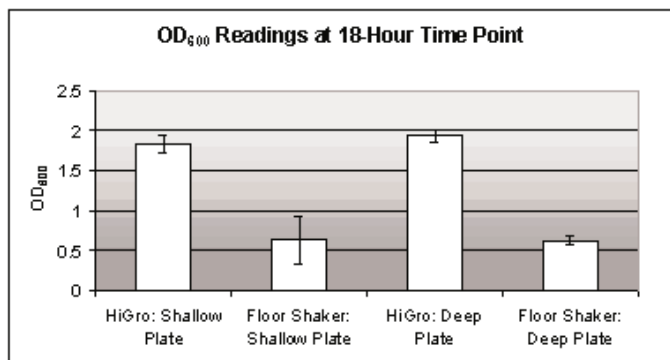
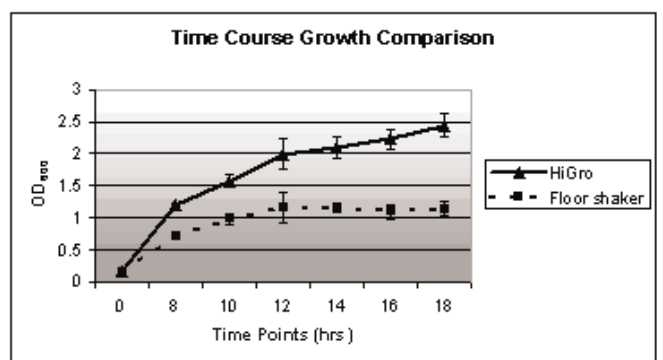
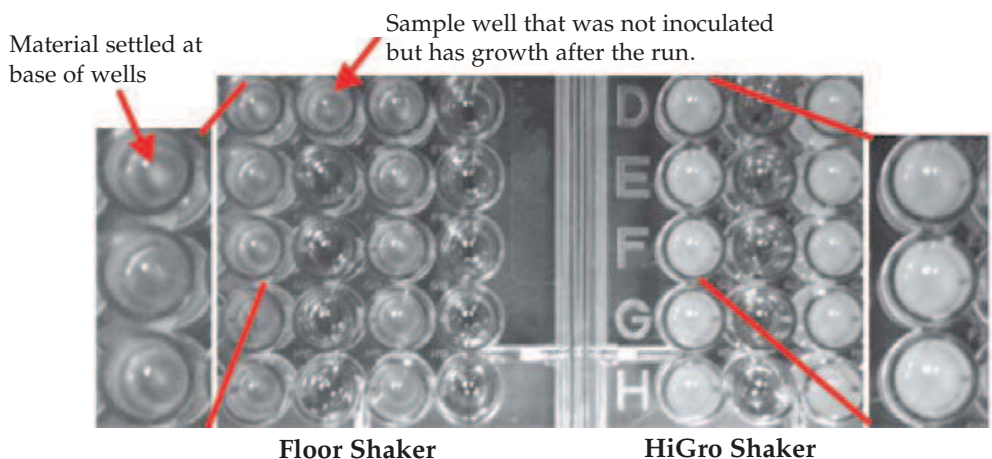


Figure 2. Time course comparison of 96-well growth densities.\*



\*Every other column was inoculated in the growth plates. OD600 readings were taken for the inoculated wells and then averaged for the entire plate.

Figure 3. Visual comparisons of sample uniformity and turbidity in 96-well shallow-well plates. Every other column was inoculated.



### 384-Well Growth

Time course results for DNA yield from 384-well growth are shown in Figure 4. Comparison of the 2 µl sample at the 8-hour time point to the mass ladder indicates that within an 8-hour growth period it is possible to obtain >10 ng of high copy plasmid DNA per 1 µl of culture. The data also show that DNA yields do not increase appreciably past the 14-hour time point.

Figure 4.

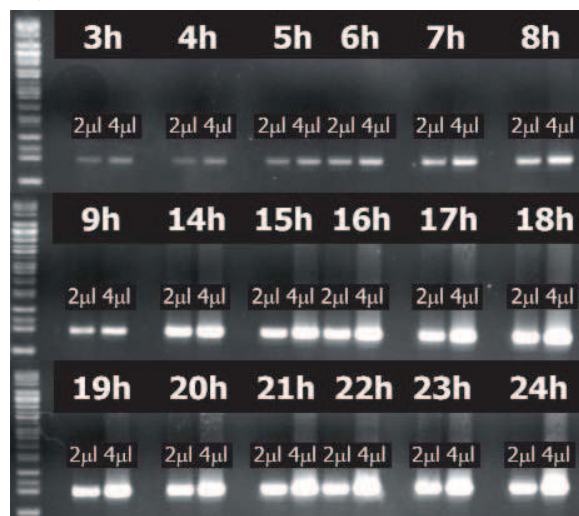


Figure 4. Time course of DNA yield from 384-well growth in the HiGro shaker.

2 µl and 4 µl of each (50 µl) DNA prep were run on a 1% agarose gel. The ladder consists of two, 10-band mass ladders combined (250 ng of each ladder). The approximate amount of total DNA from each prep can be determined by comparing the brightness/intensity of the sample bands to the ladder band that is closest in size. Based on this quantification method, the "2 µl" sample under the "8h" time point appears to be of equal or greater brightness than the mass ladder band of comparable size. This result indicates that greater than 1µg of DNA is present in the total sample volume at the 8h time point.

## ▶ Conclusions

Table 1 summarizes the steps for setting up the HiGro shaker for the growth protocols that are described in this Application Note. For either the 96- or 384-well format growth, the set-up is quick, convenient and reproducible from run to run. Furthermore, with unique features such as a small shaking orbital, gas flow and high shaking speeds, the HiGro can optimize growth conditions for higher yields and shorter growth run times. As shown in the 96-format data comparison, the growth rates and efficiencies in the HiGro can be greatly increased over those in a standard floor shaker. In 384-well plates, despite the reduced volume in the 384-well format, the HiGro can facilitate significant DNA plasmid yield after a very short growth period. The combination of convenience, high-capacity and unique growth controls makes the HiGro shaker an ideal solution for high-throughput, plasmid growth applications.

**Table 1. Summary of HiGro Shaker Set Up**

Step	Description	Details and/or Tip
1	Pre-heat the chamber(s)	30 minutes before loading plates, set the temperatures on the chambers that will be used.
2	Set the gas flow	<b>TIP:</b> After finding the right gas settings, leave the regulator, flowmeter, valves and timers at those settings. To turn the gas flow on/off on a daily basis, use the tank valve.
3	Load the plates into the cassettes	<b>TIP:</b> If the plates do not fill up the cassette(s), place the plates into the middle shelves. <b>TIP:</b> If low humidity is a concern, place a one-well plate containing a wetted paper towel in a bottom shelf. <b>TIP:</b> If condensation is a concern, place a plate-lid in the top shelf.
4	Load the cassettes into the chambers	Remember to load a cassette into each chamber regardless of whether or not it will be used!
5	Balance the off-setting chambers with equal weights	<b>TIP:</b> For convenience, prepare some balance plates or blocks that are filled with water and sealed with aluminum foil tape. These items can be left next to the HiGro shaker and used whenever balancing is necessary.
6	Secure all plate holders and lids	Do a quick check before starting the shaking motion to make sure that all of the red knobs face downward and that no lid latches are undone.
7	Start the shaking motion	<b>TIP:</b> Once a good shaking speed has been determined for an application, mark the dial setting with tape on the front panel so that setting the shaking to the same speed every time is easy.

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\*384-well data courtesy of customer collaboration.

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