



Aliquoting Agar Containing Solutions with the MicroFlo™ Select Dispenser

Dispensing Hot Solutions

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Agar is a commonly used agent to produce a semisolid substrate for cellular growth studies. The solutions are liquefied by heat and poured or dispensed into molds where they solidify upon cooling. Traditionally petri dishes have been used as the mold of choice, but the advent of microplates has allowed the miniaturization of container. While the use of 96-well microplates has reduced the size and increased the numbers of individual experiments, the diminutive size of wells has made dispensing agar containing solutions more difficult. Here we describe the use of the MicroFlo™ Select Dispenser from BioTek Instruments to easily and reliably dispense hot molten agar solutions into 96- and 384-well microplates.

Introduction

Agar is a phycocolloid extracted from the cell walls of several species of red-purple marine algae. It is an unbranched polymer consisting of galactose subunits, that when dissolved in boiling water and cooled forms a gel. Agar is primarily used as a medium for growing bacteria, but specialized forms such as agarose can be used for gel electrophoresis. Other uses for agar include serving as thickening agents in foods and cosmetics, and for clarifying beverages.

Agar has several characteristics that make it amenable for use as bacteria media. Unlike gelatin, which is formed from collagen, bacteria do not degrade agar. In addition it is firmer and stronger than gelatin. More importantly is its melting and gelling temperatures. Agar melts at approximately 85°C, yet solidifies at 32-40°C, depending on concentration. This property, known as hysteresis, allows for agar solutions to be poured into molds at considerably cooler temperatures than required for melting. Besides making it safer to work with, this feature reduces the likelihood that the molds will deform with the addition of hot solutions. In addition, the ability to add heat-labile substances, such as antibiotics, at temperatures lower than the melting point is critical for the genetic selection of bacteria.

The MicroFlo™ Select uses a specialized peristaltic design to deliver volumes from 1 µL to 10 mL.

Three different autoclaveable cassettes (1 µL, 5 µL, 10 µL), each incorporate appropriate dimension tubing, tip properties and accurate dispensing technology to deliver full and accurate incremental volumes of liquid within its specified volume range. Cassettes and cassette tubing are easily replaceable. Up to eight distinct reagents can be dispensed at one time, with compatible vessels including 6- to 1536-well microplates, PCR trays, deep well microplates, microtubes, and many other tube configurations up to four inches in height. Priming is directed into a two-stage priming trough, where liquids can be captured for recovery or directed to waste.



Figure 1. Instrument configuration. A MicroFlo configured with a 10 µL tubing cassette.

Materials and Methods

LB powered media (L-3022) and Agar (A-1296) powders were purchased from Sigma Aldrich (St. Louis, MO). LB media was first made by dissolving powdered media at a concentration of 20 g/L. The pH was corrected to pH 7.0 and agar powder was then added to a concentration of 1.5%(w/v). In order to both dissolve the agar powder as well as sterilize the solution, the mixture was autoclaved for 20 minutes. Immediately after the completion of the sterilization cycle the mixture was moved to the MicroFlo Select for dispensing into 96- and 384-well microplates. The dispense precision and linearity was assessed by measuring the absorbance at 405 nm with a Synergy™ HT Multi-Mode Microplate Reader (BioTek Instruments).

Instrument Setup: The MicroFlo™ Select was configured with a 10 µL cassette. Immediately adjacent to the MicroFlo Select was a hotplate/stirrer. A ring stand was used to hold the tubing above the media bottle and away from the hotplate/stirrer (Figure 1). This prevented the tubing from touching the hot plate and melting, while allowing the end to be immersed in the hot solutions.

Instrument Preparation: Prior to dispensing the agar containing media, the MicroFlo™ Select was primed with copious amounts of sterile deionized water (95°C) that had just been heated to boiling on the adjacent hotplate. This served to warm the fluid lines and tubing assembly.

Instrument Cleaning: Cleaning of the cassettes was accomplished by priming large amounts of boiling water through the dispense tubes. Deionized water was heated to boiling in a microwave and the temperature maintained by use of the hotplate stirrer. The initial effluent was caught using the reagent capture trough and the remainder was allowed to flow through the waste tube to a waste bucket.

Results

Initial experiments involved determining the amount of temperature loss as a result of dispensing fluid through the cassette tubing. As expected, the greatest degree of loss resulted when the fluid was at or very near boiling (~ 95°C). Under these conditions a temperature drop of approximately 25°C was observed. When agar media was cooler (~ 50°C), the drop in temperature across tubing was only 5-6°C.

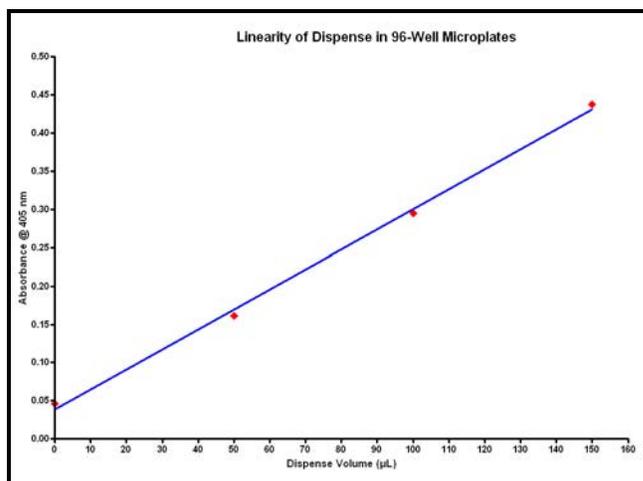


Figure 2. Linearity of Dispense in 96-Well Microplates.

After dispensing various volumes of liquid agar into 96-well microplates, the precision and accuracy was assessed by measuring the absorbance of the media in the well. Absorbance spectral scanning experiments had shown that the media had a significant absorbance at 405 nm, while the microplate demonstrated very little absorbance (data not shown). When the absorbance values for four different dispense-volumes (0, 50, 100, and 150 µL) are plotted a linear relationship is observed (Figure 2). In addition to being linear with respect to volume, the MicroFlo Select provides a very uniform dispense to all the wells. As demonstrated in Figure 3, all of the wells of a 96-well microplate have the same absorbance after 150 µL of liquid agar containing media was dispensed. This indicates that the amount of fluid in all the wells is the same.

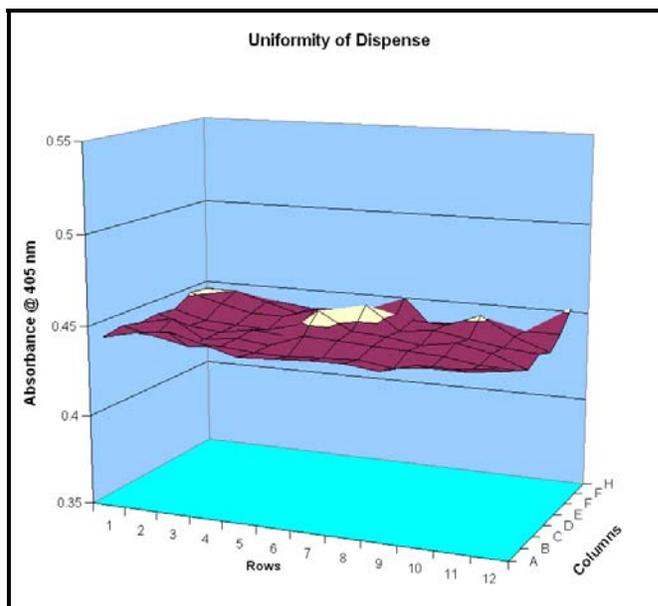


Figure 3. Uniformity of Dispense into 96-well Microplates.

When similar experiments are performed with 384-well microplates, equivalent results are observed (Figure 4). The smaller volumes necessitated by 384-well plates, along with the much smaller diameter of the wells makes the dispensing of hot liquid agar containing solutions more difficult, necessitating higher starting fluid temperatures.

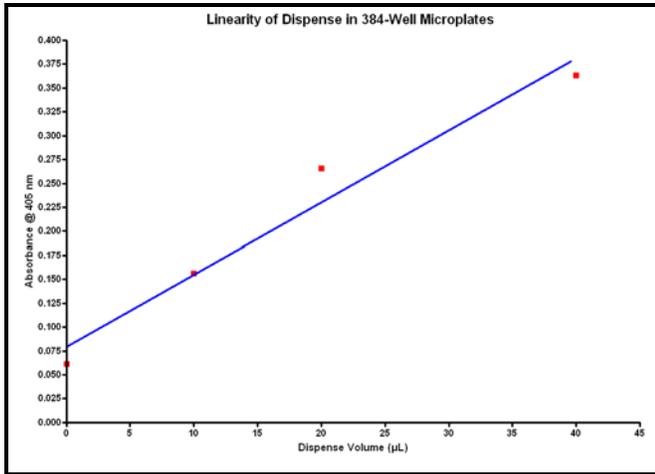


Figure 4. Dispense Linearity in 384-Well Microplates.

Discussion

These data indicate that the MicroFlo™ Select Reagent Dispenser is a suitable instrument to dispense hot liquid agar containing solution. The rapid nature of the peristaltic pump design moves the warm solution from the reservoir to the microplate without a significant loss of heat such that the solution remains in a liquid state.

Dispensing agar-containing solutions into 384-well microplates presents more challenges than 96-well plates. The small diameter and larger plastic mass, which serves as a heat sink, of the 384-well plate make it more likely to cool the agar-solution to quickly. This results in air pockets and bubbles being trapped in the well below the top surface of the agar. In order to prevent this from occurring it is advisable to use the agar solution at as high a temperature as possible. In addition, warming of the empty microplates with some sort of heat-lamp or warming oven might also be advisable to keep the agar from prematurely gelling in the microplate.

Priming of the instrument with hot water was found to be a critical element both before and after the dispensing of the agar solution. Prior to dispensing the fluid, the hot sterile water is used to warm the fluid lines, as well as the solid components of the fluid cartridge. This prevents a rapid loss of the agar containing solutions, which would result in premature gelling of the fluids in the tubing. Subsequent to dispensing of the agar solution, the hot water serves to dissolve any residual agar remaining in the tubing, making it much easier to clean and re-use the cassette. The use of copious amounts of hot water before and after dispensing requires that the reagent recovery trough be removed during these priming procedures. However, it is advisable to use this recovery trough to catch the much smaller amounts of agar containing liquids that are dispensed during the plate primes. This material will eventually gel as it cools and

cleaning the fluid recovery trough is significantly easier than cleaning the drain tubing associated with the catch basin.

There are many features of the MicroFlo Select that make it conducive to dispensing sterile temperature-dependent solutions such as agar mixtures. The small size of the MicroFlo and a standard hotplate-stirrer allows the entire setup to be placed into a standard biosafety cabinet. This would provide a sterile environment in which to dispense into microplates, preventing possible contamination by airborne bacteria. The low heat sink mass of the cartridge and peristaltic pump reduces temperature loss during the dispense procedure. As a result, a high temperature is not required to maintain a liquid state of the solution. This makes the MicroFlo Select more amenable to heat labile compounds, such as antibiotics, that can only be added to the heated solution after the solution has been cooled considerably. The MicroFlo Select utilizes eight separate multiple tubes to dispense fluids. Using the included tubing weight, all eight tubes are easily managed with a single solution. By separating the tubing from the weight device, each tube can be placed into a different reagent reservoir. This means that up to eight different solutions can be dispensed simultaneously, allowing multiple antibiotic bacterial selections to be performed on a single microplate.

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