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Application Note

Monitoring the Growth of *E. coli* With Light Scattering Using the Synergy™ 4 Multi-Mode Microplate Reader with Hybrid Technology™

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Monitoring cell growth is an important tool in the biological sciences. Microplate readers with temperature control can process large numbers of samples and document the effects of growth inhibition. Here we describe the use of the Synergy™ 4 Multi-Mode Microplate Reader to monitor the growth of E. coli and the inhibition by ethanol at a variety of wavelengths.

Introduction

Quantitation of bacteria and yeasts is an important tool in molecular biology. In particular, the ability to transfer plasmids into E. *coli* enables production of recombinant proteins. Endpoint quantitation of E. *coli* is used to normalize results to a standard amount of bacteria and inhibitory effects can be studied via growth studies. Absorbance at 600 nm is the recommended wavelength for measuring solutions containing E. *coli* or other bacterial samples.

Research citing the use of microplate readers measuring cell growth at 600 nm is plentiful. Stubbings, *et al*, validated microplate reader 600 nm cell growth reading compared to classical methods for studying post antibiotic effects [1], while Shapiro, *et al*, used lysogen growth measurement at 600 nm to develop a means to detect sublethal concentrations of antibacterial agents that can interfere with prokaryotic translation [2]. Brocklehurst, *et al*, examined the metal-ion tolerance of various strains of E. coli by determining the MICs of each culture at 600 nm in the presence of several metals after a 24-hour period [3]. Chekmenev, *et al,* studied the amphipathic cationic antimicrobial peptides from fish, amidated and non-amidated piscidins 1 and 3, using a modified liquid growth assay, which was read at 600 nm and the MICs reported [4]. Lauth, *et al,* isolated a novel antimicrobial peptide, moronecidin, from the skin and gill of hybrid striped bass and the antimicrobial properties were monitored with a modified liquid growth assay read at 600 nm [5]. Blanchard, *et al,* studied OxyR and SoxRS systems in *E.* coli co-regulated SoxRS-dependent and independent genes modulated by superoxide minutes after exposure to stress.

The above research notes that while a variety of absorbance readers can read bacterial endpoint and growth curves at 600 nm, maintaining a uniform 37°C is recommended for reading the changing absorbance of the growth of E. *coli* over long periods of time eliminating variations due to room temperature changes.

BioTek markets a variety of readers with the capability of monitoring growth curves at 600 nm with temperature control at 37°C including the new Synergy 4 Multi-Mode Microplate Reader with Hybrid Technology, the Synergy 2 and Synergy HT Multi-Mode Microplate Readers, the PowerWave series of monochromator based microplate readers and the ELx808IU filter based microplate reader.

This application note demonstrates the capability of a BioTek plate reader to reading an E. *coli* growth assay and to illustrate the effect of a growth inhibitor such as ethanol. The experiment below uses the Synergy 4 Multi-Mode Microplate Reader with Hybrid Technology which combines a quad monochromator system with high powered xenon lamp for flexible wavelength selection with another optical system featuring a quartz halogen lamp and filter wheel system for assays requiring the most sensitive detection limits including fluorescent proteins, time resolved fluorescence, the demands of FRET and TR-FRET, fluorescence polarization, ratiometric ion channel assays, filtered luminescence such as BRET and BRET2 and AlphaScreen®. The experiment below uses the third optical system in the Synergy 4, a super quiet xenon flash lamp with dedicated monochromator for absorbance reading.

Materials and Methods

The bacteria used were E. *coli* strain DH5α. LB broth part number L3022 was obtained from Sigma-Aldrich (St. Louis, MO) and made as directed. Reagent alcohol (100%) part number A962-4 was obtained from Fisher Scientific. Sterile covered microplates part number 3603 and UV transmissible microplates

part number 3635 were obtained from Corning Life Sciences. The Synergy 4 Hybrid reader controlled by Gen5™ Software was supplied by BioTek Instruments, Inc. Samples were pipetted in replicates of 8, with each strip being a different experimental parameter. Columns 1 through 6 contain decreasing amounts of ethanol per the following table. Columns 7 through 12 contained 195 µL of media and 5 µL of bacterial culture.

LB broth (µL)	Ethanol (µL)	% Ethanol	E. *coli* (µL)
115	80	40	5
155	40	20	5
175	20	10	5
185	10	5	5
190	5	2.5	5
192.5	2.5	1.25	5

The following procedure was programmed into the reader:

1. Set temperature at 37°C.
2. Withdraw the plate into instrument for a 10 second shaking at medium speed.
3. Read the plate every 5 minutes for 24 hours at the following wavelengths: 340 nm, 570 nm, 600 nm, 630 nm, 800 nm and 850 nm while maintaining the temperature at 37°C.
4. Shake when not reading (continuous setting) at slow speed.

After reading, the contents of one well was transferred to a well on a Corning 3635 UV plate and scanned from 200 to 999 nm in 1-nm increments (Figure 3).

Results

Wells untreated with ethanol showed a uniform increase with a flattening beginning by 0.8 OD as typified by the well below (Figure 1).

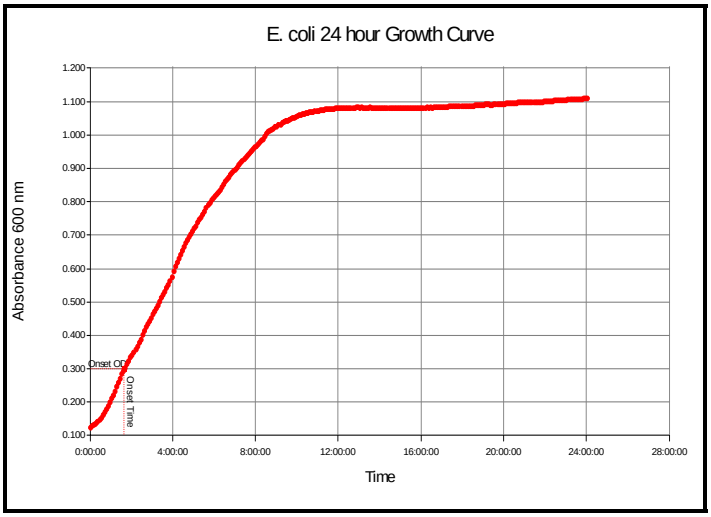

Figure 1. Typical E. *coli* Growth Curve. The plates was read every 5 minutes for 24 hours at wavelengths of 340, 570, 600, 630, 800, and 850 nm with shaking before each cycle. The above graph depicts a typical growth curve at 600 nm.

Effects of Ethanol Inhibition

As documented in Figure 2 and the table below, increasing dilutions of alcohol were pipetted in columns 1-6 with column 7 being the first column containing no alcohol. Note that column 1 has the most alcohol and no evident growth.

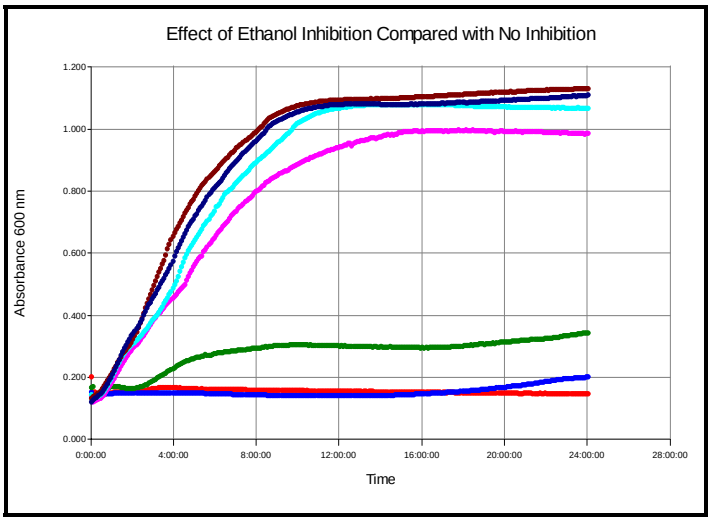

Figure 2. Comparison of ethanol concentration on growth curves.

Color	Ethanol (%)
Red	40
Blue	20
Green	10
Fuchsia	5
Turquoise	2.5
Dark Blue	1.25
Brown	0

Using different wavelengths for reading cell growth

Reading at 600 nm is the traditional wavelength for cell growth. However, because the absorbance noted at 600 nm is due to light scattering and because not all plate readers are equipped with a 600 nm capability, a scan of the contents of H-12 was performed to see what effect various wavelengths had on the results. The contents were transferred to a Corning 3635 UV transmissible microplate to determine if 600 nm featured a unique peak, if other wavelengths could be used, and if pathlength correction to 1 cm could be used if necessary. As noted in Figure 4, there is nothing unique regarding the 600 nm wavelength used as compared to other wavelengths. When other wavelengths are used a very similar shaped curve is observed. Theoretically, a whole variety of wavelengths could be used to measure cell growth. Note that at lower wavelengths, (e.g. 340 nm) other reagents, such as the microplate itself and dissolved solutes also absorb light and these wavelengths are not suitable for measuring light scattering. Reading at 600 nm is still the traditional wavelength however, and the one cited in the literature.

In order to directly compare the results of bacterial growth curves in microplates to those obtained in spectrophotometer cuvettes a correction for pathlength is required. Automatic BioTek pathlength correction depends on the solution having an isolated absorbance peak for the diluent. In the case of aqueous solutions, water has a peak at 977 nm. A reading at 900 nm is used to subtract nonspecific absorbance effects such as plastic. Unfortunately, this method of pathlength correction cannot be used because the actual measurement of bacterial growth is based on light scatter rather than true absorbance. As demonstrated previously, these experiments scatter light all wavelengths including 977 nm and 900 nm, which affect the pathlength correction calculations. At 977 nm, with 200 µL in the well, the absorbance of 200 µL of aqueous solution should be around 0.100 above the absorbance at 900 nm. Instead, the

respective absorbance values are 0.883 and 0.870 for 977 and 900 nm respectively, a difference of 0.013 which indicates other reagents are absorbing and or scattering light at those wavelengths, a condition that negates the use of that method of pathlength correction. A more effective method of pathlength correction involves the determination of the pathlength of a specific volume of fluid using water or absorbing colored dye prior to running a bacterial growth curves. This calculated pathlength could then be used as a constant in a mathematical transformation to correct each well to a 1-cm pathlength value.

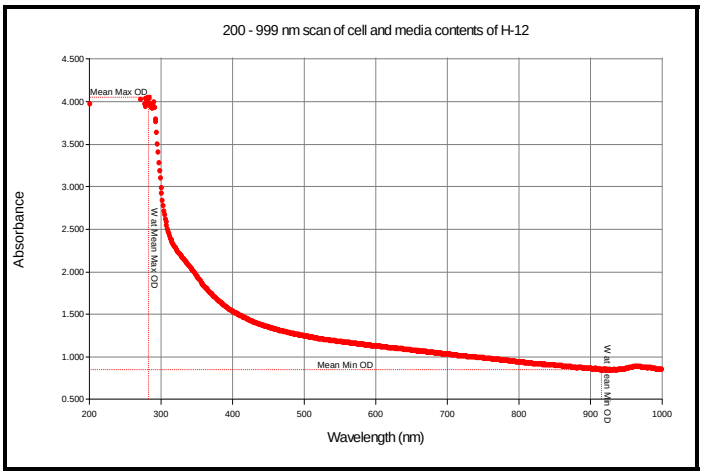

Figure 3. Spectral scan of DH5- E. *coli* bacteria and media contents. The contents of a cell growth assay plate were transferred to a Corning 3635 UV transmissible plate and scanned from 200 nm to 999 nm in 1 nm increments using a Synergy 4 Multi-Mode Microplate Reader with Hybrid Technology.

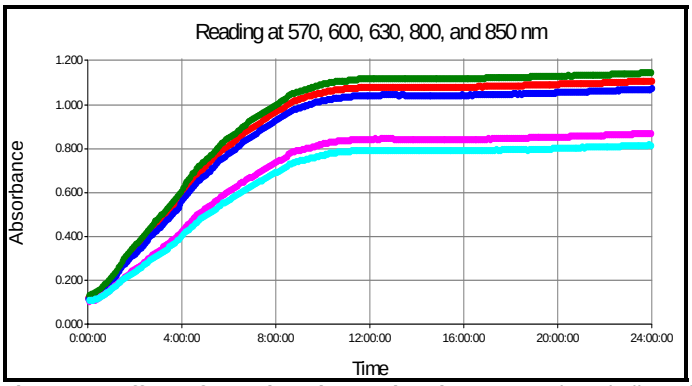

Figure 4. Effect of wavelength on Absorbance. Various indicated wavelengths were used to assess bacterial growth over time and plotted using Gen5 Data Analysis Software. Each well was read with 7 wavelengths including 570, 600, 630, 800, and 850 nm as displayed in the table above.

Wavelength	Graph color
570 nm	Green
600 nm	Red
630 nm	Dark blue
800 nm	Fuchsia
850 nm	Turquoise

Discussion

These data demonstrate that the BioTek Synergy 4 Multi-Mode Reader with Hybrid Technology is capable of monitoring cell growth at a variety of wavelengths including the traditional wavelength at 600 nm. Growth is dependent on media and a lack of inhibition. Growth can also be monitored at a variety of wavelengths so that results are not restricted to 600 nm.

References

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Rev. 1/9/08