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Monitoring Cell Growth in 2 µL Volumes Using a Microplate Reader

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Accurate determination of cellular growth in culture is critical to many downstream processes to insure optimal growth is achieved and sufficient biomass is being produced. The diverse nature of cell types currently used in scientific processes requires a universal method for monitoring cell growth. A common and rapid method for determination of cell growth is the use of turbidimetry. Here we describe a suitable method of performing micro-volume cell quantification for a variety of cell types from as little as 2 µL of cell culture using a microplate reader.

Key Words:

Micro-Volume Analysis

Cell Quantification

Turbidity

Cell Growth

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Introduction

Cell culture is an important process for growing a diverse range of cell types. Commonly used types include bacteria, yeast, and mammalian cells. These provide the starting material for such diverse processes as the production of biologics, alcoholic beverages and in-vitro model systems for drug discovery and toxicological studies. While the strains and cells types being investigated may differ broadly, turbidimetry remains a common and rapid method to measure cell growth. Typically optical density measurements are made at a wavelength of 600 nm using cuvette- or microplate-based readers. The choice of wavelength is due to the fact that many of the components that comprise cell culture media remain transparent at this wavelength so that optical density is proportional to the density of cells in the light path. While turbidimetry does not follow Beer's law and thus is not necessarily linear, calibration curves can be reliably generated[1]. A calibration curve from a single cell type can then be extrapolated and provides a basis for quantitative analysis of cellular growth by sampling a portion of the propagating culture.

Materials and Methods

Daudi Cells

The human peripheral Daudi cells were obtained from ATCC (P/N CCL-213, Manassas, VA, USA). RPMI medium, nonessential amino acids (NEAAs), fetal bovine serum (FBS), penicillin/streptomycin/glutamine (P/S/G) and DPBS buffer were obtained from Invitrogen (Carlsbad, CA). Daudi cells were maintained in RPMI supplemented with 10% FBS, 1X NEAA and 1X P/S/G as a suspension cell culture and split 1:10 v/v every 2-3 days. The cells were harvested as needed by centrifugation. Cell number was determined by using a hemacytometer. A 1:2, 8-point serial dilution was performed in DPBS for turbidimetry measurements. Samples were measured in duplicate at a wavelength of 600 nm using the microspots of the Take3™ Trio Micro-Volume Plate on an Eon™ Microplate Spectrophotometer (BioTek Instruments, Inc, Winooski, VT). The reader was controlled and data collected using Gen5™ Data Analysis Software (BioTek Instruments, Inc, Winooski, VT).

The phase contrasted brightfield image of 2,000 Daudi cells in a microspot of the Take3 Trio plate was taken with a 4x microscope objective.

Yeast

YPD media powder, sodium chloride, and monobasic and dibasic phosphate were obtained from Sigma-Aldrich (St. Louis MO). YPD media was prepared as directed and sterilized by autoclaving. The yeast strain was obtained from Wyeast Laboratories (Odell, OR). Overnight stock cultures (50 mL) were grown in 250 mL Erlenmeyer flasks at 30°C with orbital shaking at 125 RPM. The overnight stock was used for either determination of cell number using a hemacytometer or to create a 1:2, 10 point serial dilution in YPD for turbidimetry measurements as described above.

Bacterial Cells

E. coli variant JM109 was obtained from Promega (Madison, WI). Either the parent strain or E. coli transformed with plasmid pTRACER-CMV2 (Invitrogen, Carlsbad, CA) were grown in culture as described below. Luria Bertani (LB) broth was obtained from Sigma-Aldrich (St. Louis, MO), prepared as directed and sterilized by autoclaving. Overnight stock cultures were incubated overnight at 37°C with shaking (5 mL) in 15 mL conical tubes. The overnight culture was used for determination of cell number by use of the Miles and Misra method to determine the colony forming units (CFUs) in the bacterial suspension by plating 50 µL of a 1:100 serial dilution series on LB/agar plates . A 1:2, 8 point serial dilution series was generated from the overnight stock for turbidimetry measurements at a wavelength of 600 nm. The overnight culture was also used for inoculation of cultures for growth studies. Cultures inoculated for growth studies used a 50:1 v/v dilution of LB to overnight culture. Aliquots of the culture were measured at the appropriate time intervals in either a low-volume 1 cm pathlength disposable cuvette or using the microspots of the Take3™ Micro-Volume Plate on an Eon™ Microplate Spectrophotometer equipped with a cuvette port as described above.

HepG2 Cells

The human cell line HepG2 was obtained from ATCC (P/N HB-8065). DMEM, glutamine, fetal bovine serum (FBS), P/S and DPBS buffer were obtained from Invitrogen. HepG2 cells were maintained in DMEM supplemented with 10% FBS, and 1% P/S. The cells were harvested at confluency by trypsin digestion and resuspended in DPBS buffer. Cell number was determined by using a hemacytometer. A 1:2, 8-point serial dilution was performed in DPBS for turbidimetry measurements as described above.

Results

Calibration curves for different cell types are necessary for accurate determination of cell density due to differences in their ability to attenuate light. Optical density at 600 nm was used to construct calibration curves for Daudi, yeast, bacteria, and HepG2 cells (Figure 1). We have chosen to show the data as the number of cells in the microspot of the Take3 Trio Plate to demonstrate how few cells are required for accurate measurement. Figure 2 shows an image of a single microspot of the Take3 Trio Plate which has approximately 2,000 Daudi cells in the 2 µL volume (data point highlighted in Figure 1A). The curves in Figure 1 can be used for the determination of cell density during growth of the various cell types in culture.

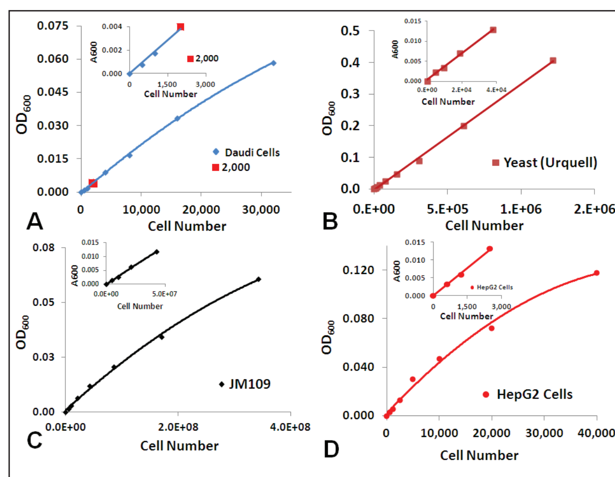

Figure 1. Calibration curves for various cells types derived from measurement of changes in optical density induced by turbidimetry at 600 nm a) mammalian Daudi cells, b) yeast c) bacterial E. coli strain JM109 and d) HepG2.

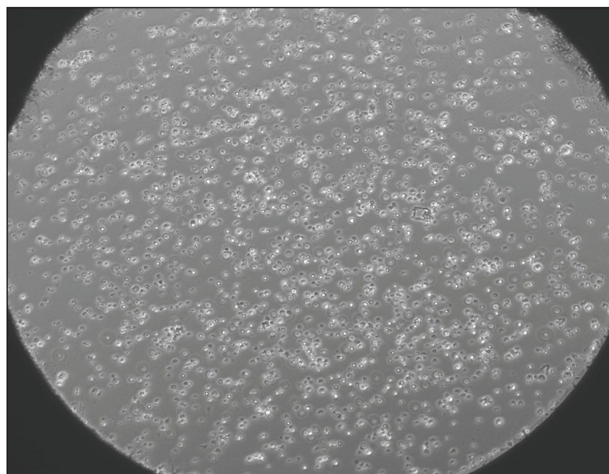

Figure 2. 2,000 Daudi cells in a 2 mm microspot of the Take3 Trio plate yielding an optical density of 4 mOD. Depth of the image is 0.5 mm – some cells are in focus, others out of the focal plane.

Cell growth is typically monitored to assure the appropriate cell density is achieved prior to performing additional manipulations such as induction of protein over-expression in bacterial cells or determination of optimal growth conditions during assay development. Growth of JM109 cells in culture was monitored by removing a sample at 60 minute intervals for 10 hours and reading optical density at 600 nm using either the micro-volume Take3 Trio accessory plate or low-volume cuvette (Figure 3). Superimposition of growth curves shows excellent correlation between the two analytical methods. The typical stages of bacterial cell growth in culture are observed: lag, log and stationary phases at early, middle and late time points, respectively. This plot demonstrates the ability to accurately monitor bacterial concentrations within the range of 10^8 – 10^9 bacterial cells/mL (0.8-1.0 OD in a cuvette,1 cm pathlength; equivalent to 60-70 mOD in the Take3 Trio Plate, 0.5 mm pathlength) typically associated with addition of induction reagents for protein expression systems.

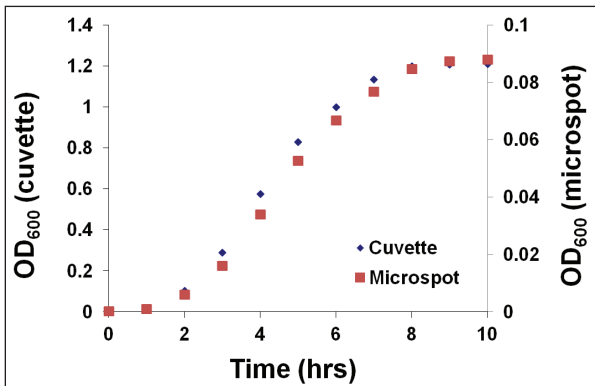

Figure 3. Growth curves of JM109 depicting hourly A600 measurements using either a) 2 µL sample on Take3™ Micro-Volume Plate or b) 500 µL in a low-volume cuvette.

Conclusions

Turbidimetry is a popular method for rapid cell growth analysis. Its sensitivity coincides with useful cell densities for downstream applications. The use of the micro-volume format with as little as 2 µL allows for rapid analysis and sample preservation to be realized. The Take3 Trio in conjunction with the Eon™ Microplate Spectrophotometer with optional cuvette port is a flexible system that can accurately monitor cell growth over a wide range of cell types.

References

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