APPLICATION NOTE

Measure long-term cell growth using a discontinuous kinetic reading

Introduction

Many biological experiments require monitoring cell growth or measuring enzymatic changes over long periods of time (hours, days or even weeks). In addition, certain model organisms cannot be viably kept in the microplate reader for long periods of time. Algae cultures, for example, require periodic exposure to light and high humidity levels in order to grow successfully, and comparative studies of algae growth conditions are measured over the course of weeks[1]. Long-term kinetic measurements can also be used to study other facets of biology such as enzyme kinetics, cellular signaling, and protein expression. However, running such an assay as a long-term continuous kinetic reading on a microplate reader involves occupying the reader for an extended period of time, greatly reducing a lab's efficiency. Additionally, making a series of endpoint reads yields a data set that must be manually linked together to make a kinetic plot.

Using Molecular Devices microplate readers and SoftMax® Pro Software, researchers can now take measurements over long periods of time using the software's Interrupt and Append features (Figure 1). These features allow the removal of the microplate from the instrument for media additions or other experiments and then resume a kinetic reading while keeping all the data points in a single plate section for ease of analysis.

In this application note, we demonstrate how to measure bacterial cell growth via absorbance at 600 nm (reported as OD_{600}) over the course of 24 hours using a discontinuous kinetic reading protocol on the SpectraMax® iD3 Multi-Mode Microplate Reader.

Materials

- 96-well solid bottom, clear polystyrene microplates (Greiner cat. # 651101)
- BactoBeads™ *E. coli* GFP Host (Edvotek cat. #728)
- Lysogeny broth (LB, Sigma Aldrich cat. #L2542)
- Ampicillin (Sigma Aldrich cat. #A0166-5G)
- SpectraMax iD3 Multi-Mode Microplate Reader (Molecular Devices cat. #iD3)

Methods

An ampicillin-resistant *E. coli* stock with an OD_{600} value of 0.32 was diluted 10-, 100-, and 1000-fold in 10 mL LB containing 100 µg/mL ampicillin. Over a period of 24 hours, changes in OD_{600} were measured by periodically transferring 100-µL aliquots of each bacterial culture to a single well of a 96-well microplate and measuring absorbance at 600 nm using the SpectraMax iD3 reader and SoftMax Pro Software. Instrument settings are listed in Table 1.

Benefits

- **Improve lab efficiency by freeing up the instrument between kinetic measurements**
- **Automatically link together several data points into a kinetic graph**
- **Compare different growth conditions using SoftMax Pro Software**

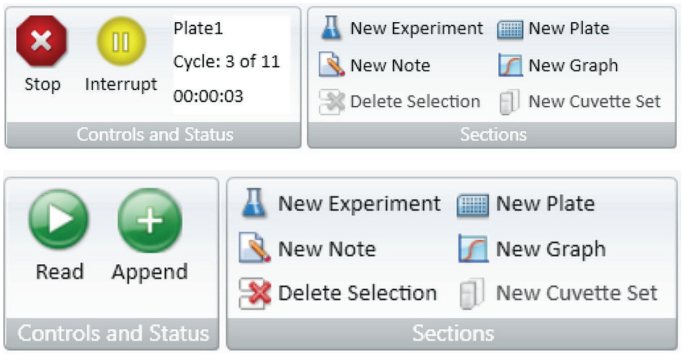

Figure 1. Appending kinetic measurements. Kinetic measurement can be interrupted by pressing the Interrupt button during the measurement in the Controls and Status panel. To resume collecting kinetic measurements within the same file, press the Append button.

A kinetic plate read was initiated at time zero. To interrupt the kinetic measurement, the "Interrupt" button was clicked in the "Controls and Status" section of SoftMax Pro Software. The instrument finished reading the remaining wells in the selected read area before pausing. The plate was then removed from the reader. For the next time-point in the kinetic reading, a new set of samples was taken from the cultures, and the plate was inserted into the reader. The original data file was opened, and the "Append" button was clicked to enable the addition of more data points to the kinetic reading (Figure 1). The Append feature linked together the discontinuous measurements into one continuous bacterial growth curve. The onset time of the bacterial growth curves was calculated in SoftMax Pro Software to compare bacterial growth conditions. A data reduction was set up to calculate the onset time required for the bacterial culture to reach 0.6 OD_{600} and to calculate the slope at the inflection point of the bacterial growth curve (Table 2).

Results

Bacterial cultures progress through four phases: lag phase, log phase, stationary phase, and death phase[2] (Figure 2). We collected enough data points to generate bacterial growth curves from the three different bacterial cultures described in the Methods section (Figure 3). The data show each culture progressing through the lag, log, and stationary phases at different times. The death phase is not observed in the graph, because the light-scattering method measured by the instrument is more related to the mass of the sample rather the individual cells. The dead cells still occupy mass in the sample and contribute to the light scattering and, therefore, the OD_{600}.[3, 4]

Various parameters of cellular growth can be calculated using SoftMax Pro Software. Calculating the onset time when a bacterial culture reaches a certain optical density allows us to quantitatively compare growth rates. In our example, we observed the quickest onset time in the 10-fold diluted culture (Table 2). We predicted that the less diluted cultures would have the shortest onset time, and the analysis supported it.

Read mode settings	
Read mode	Absorbance
Read type	Kinetic
Wavelength	600 nm
Timing	1 reading/minute for 10 minutes

Table 1. Plate reader settings used for data acquisition of bacterial growth curve.

Bacterial dilution	Onset time (minutes)	Slope at inflection point
10-fold	1022	1.93E-05
100-fold	1118	2.24E-05
1000-fold	1175	2.46E-05

Table 2. Different parameters of bacterial cell growth. SoftMax Pro Software can be used to analyze different areas of bacterial growth. The onset time was calculated when the bacteria reached 0.6 OD_{600}.

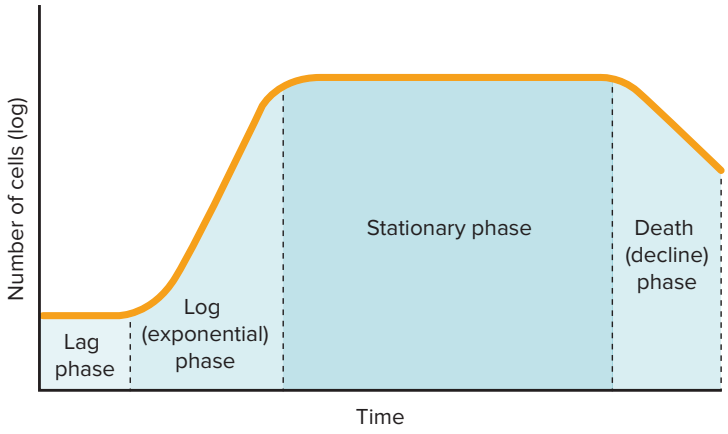

Figure 2. Bacterial growth phases. Representation of four different phases of bacterial growth.

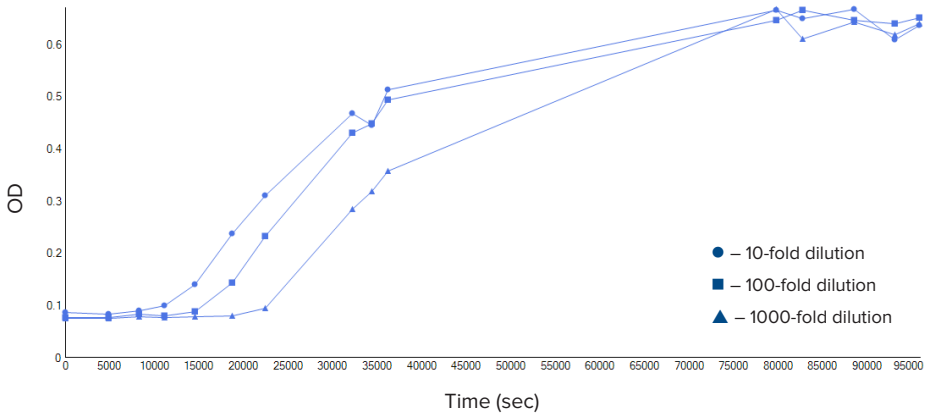

Figure 3. Bacterial growth curves. Three different bacterial dilutions were measured using the SpectraMax iD3 Reader and SoftMax Pro Software. Measurements were taken over a 24-hour period. Note that the software was still able to link data points separated by a large time gap.

The slope at the inflection point of the growth curve, which reflects the period of exponential growth, can also be calculated using the data reduction features in SoftMax Pro Software (Table 2).

Conclusion

Experiments that involve monitoring cellular growth can vary greatly in length, and it is unrealistic to expect one experiment to occupy a plate reader for multiple hours or days. With the discontinuous kinetics feature in SoftMax Pro Software, researchers can perform kinetic measurements without having to continuously occupy their microplate reader. In addition, sensitive cell types requiring strictly controlled environmental conditions can be measured without impacting cell health or survival. Finally, the formulas and data reduction can be set up within SoftMax Pro Software to perform various analyses in order to quantitatively compare different growth conditions. Assistance with formula writing can be obtained by visiting our Knowledge Base site (http://mdc.custhelp.com/) or by contacting tech support.

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