bacterial growth

stratus plate reader

introduction

Microplate readers are important laboratory tools for optical measurements of biological samples. Common applications include measurements of bacterial growth, cell proliferation, cytotoxicity, and protein concentration, as well as for ELISAs and reporter gene assays. The Stratus, a small LED-based plate reader developed by Cerillo, is uniquely versatile in its combination of small footprint, precision, and usability. This application note describes the performance of the Stratus when used for the measurement of bacterial growth.

bacterial growth

The Stratus was used to compare the growth of *E. coli* K-12 in Lysogeny Broth (LB) to *E. coli* K-12 grown in M9 minimal media. LB, a nutritionally rich medium for bacterial culture, is widely used for its high bacterial yield and convenience [1]. M9 media differs from LB media in that it allows for the customization of nutrient concentrations, thereby enabling the role of specific components to be studied.

For this experiment, LB media contained 2.5% LB powder in diH_2O. The M9 media contained M9 salts (5X), 0.4% Glucose, 0.2% 1 M $MgSO_4$, and 0.01% 1M $CaCl_2$ in diH_2O [2]. Cultures of *E. coli* were grown in LB and M9 media overnight. After 16 hours of growth, the optical density (OD) was measured for both cultures and they were diluted with the sterile growth media to a starting OD of 0.01.

The diluted growth cultures were then pipetted into a microplate, 200µL per well. The plate had 40 wells of samples from each media type, and 8 negative controls for each media type. The negative controls contained 200µL of sterile growth media. To start the experiment a "Time Course" was set up using the Cerillo Device Manager. The setting were customized by the user to read the plate every 3 minutes (Figure 1).

Due to the Stratus microplate reader's small size and versatility, it was placed inside of a shaking incubator at 37°C and 180 rpm. Data was collected for 24 hours with no further user input

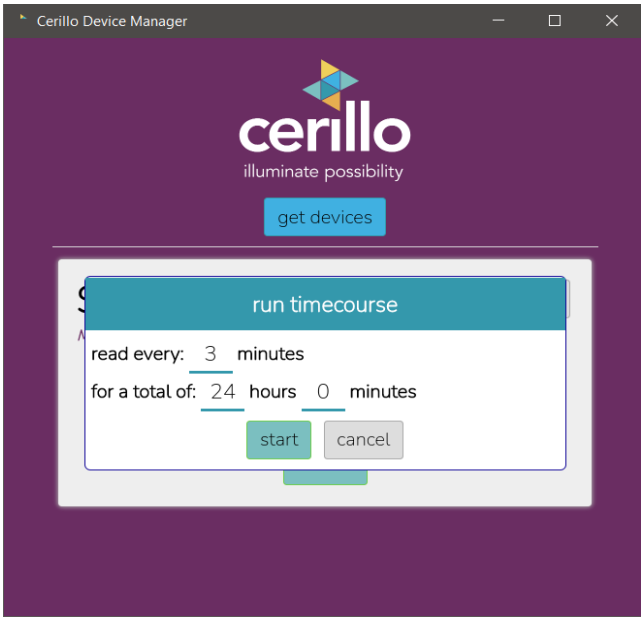

Figure 1. Time Course Setup makes it easy for the user to customize measurment intervals.

After the Time Course ended, the data was retrieved from the "files" menu on the Cerillo Device Manager and analyzed via GraphPad Prism (Figure 2). A comparison between growth curves shows that LB media allows for robust E. coli growth over a 24-hour period when compared to M9 minimal media (Figure 3). At 5 hours, the *E. coli* grown in LB media experienced a diauxic shift, where growth slowed due to the depletion of glucose and then shifted to metabolizing galactose [3]. High-frequency monitoring, demonstrated here using the "Time Course" function, enables metabolic activity to never be missed due to less frequent measurements.

Furthermore, traditional plate readers have been shown to have poor temperature regulation which causes high variability and less accuracy [4]. The use of the Stratus inside of a shaking incubator allows for the stability of environmental conditions leading to higher quality data collection.

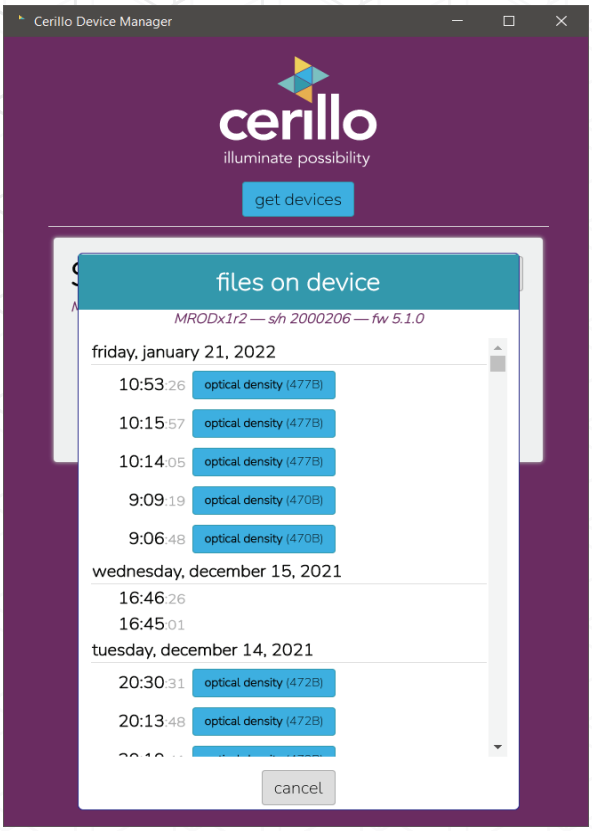

Figure 2. File Storage is easily accessed via the Cerillo Device Manager which allows data to be exported and saved for analysis.

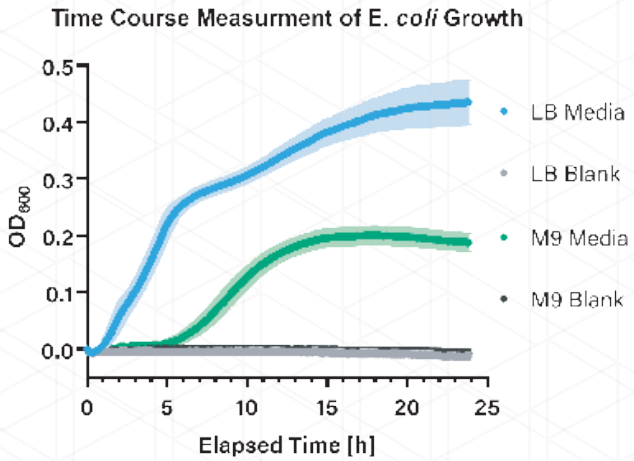

Figure 3. Time Course Measurement of *E. coli* K-12. The growth of E. coli was compared between M9 minimal media and LB Media. The growth was monitored over 24 hours at 37°C.

summary

Monitoring cell growth with the Stratus is easily performed using Time Course reading. The Cerillo Device Manager makes it simple to set up and measure a microplate with minimal hands-on time. This allows the user to spend less time on the setup and repetitive analysis leading to greater productivity. The small size and adaptability of the Stratus makes it easy for experiments to take place in nearly any environment under any conditions.

references

1. Sezonov, G., Joseleau-Petit, D., & D'Ari, R. (2007). Escherichia coli physiology in Luria-Bertani broth. Journal of bacteriology, 189(23), 8746–8749. doi:10.1128/JB.01368-07

2. Anonymous. (2010) M9 minimal medium (standard). Cold Spring Harb Protoc https://doi.org/10.1101/pdb.rec12295.

3. Siegal M. L. (2015). Shifting sugars and shifting paradigms. PLoS biology, 13(2), e1002068. doi:10.1371/journal.pbio.1002068

4. Grosch, J., Sieben, M., Lattermann, C., Kauffmann, K., Büchs,J. and Spieß, A.C. (2016), Enzyme activity deviates due to spatial and temporal temperature profiles in commercial microtiter plate readers. Biotechnology Journal, 11: 519-529. doi:10.1002/biot.201500422

Ready to get started?

Request a Quote