Comparing Microvolume and Cuvette Based Measurements of Microbial Cell Cultures

Introduction

Using a spectrophotometer, such as a Thermo Scientific™ NanoDrop™ One^{C} Spectrophotometer, to measure the optical density at 600 nm (OD_{600}) is a central technique in microbiology to monitor bacterial culture growth. Optical density measurements, however, often contain very little true chemical absorbance. Instead, optical density measurements are representative of the amount of light scattered away from the instrument's detector by the bacterial suspension. The pedestal and cuvette options found on a NanoDrop One^{C} spectrophotometer will give different OD values when measuring light scattering samples. There are two important aspects to consider when making light scattering measurements: 1) the linear range of the measurement systems; and 2) the use of a conversion factor for the two measurement systems.

Linear Range Determination

When measuring OD values of scattering samples using a spectrophotometer, it is necessary to determine the linear range, as this may not be the same range as that observed for traditional absorbance measurements. Establishing the linear range is important because the OD_{600} may exceed the linear range of the instrument prior to a cell culture reaching stationary phase. In Figure 1A, the OD_{600} of a polystyrene bead standard diverges between two dilution series at 2.11 A, indicating the upper limit of the linear range of the cuvette option on the NanoDrop One^{C} spectrophotometer has been met.

The linear range depends largely on the optical configuration, and therefore will not be the same for the pedestal and the cuvette on a NanoDrop One^{C} spectrophotometer. Note in Figure 1B, the variable pathlength technology enables measurements up to 550 A, indicating that the pedestal's linear range has not yet been reached.

Thermo Scientific™ NanoDrop™ One/One^{C} Spectrophotometer

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To determine the linear dynamic range for cell culture measurements, a series of dilutions can be performed with a young overnight culture (~16 hours) of the microbial strain and the measured OD_{600} can be graphed against the dilution factor. The limits of detection can be determined by identifying the OD_{600} at which there ceases to be a linear correlation between the dilution factor and the measured OD_{600}.

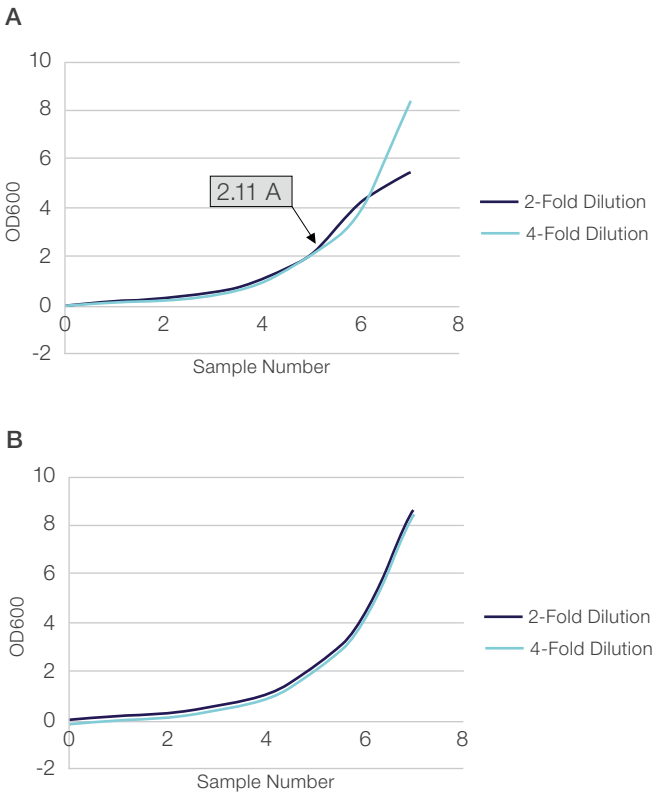

Figure 1. Polystyrene beads diluted 2-fold (dark blue) and 4-fold (light blue) and standardized to the dilution factor measured with the cuvette option (A) and the pedestal option (B) on a NanoDrop One^C spectrophotometer. The divergence shown in (A) between the two lines occurs at 2.11 A, where the sample exceeds the linear range of the spectrophotometer.

Measurement Conversion

To compare OD readings between the pedestal and cuvette systems found on a NanoDrop One^C instrument, a conversion factor can be calculated as follows:

$$\frac{\textit{Cuvette OD}}{\textit{Pedestal OD}} = \textit{Conversion Factor}$$

This conversion factor can then be used to compare measurements using cuvette and pedestal options (Figure 2). The method for calculating a conversion factor can also be used when comparing OD_{600} measurements between different spectrophotometers.

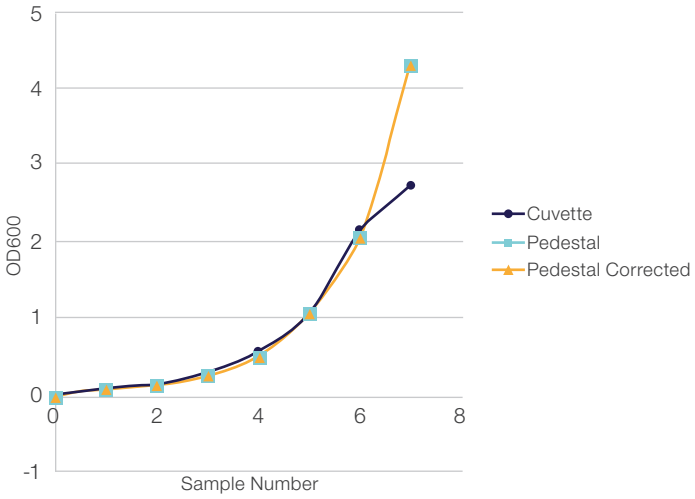

Figure 2. A dilution series of polystyrene beads using the NanoDrop One^C pedestal option (light blue squares) and cuvette option (dark blue circles). Applying the conversion factor to the pedestal data (yellow triangles) facilitates comparison between the two measurement methods.

Summary

When comparing the OD_{600} measurements made using the cuvette and pedestal options on a NanoDrop One^C spectrophotometer, the following points should be remembered:

- Ensure the OD_{600} at the bacterial growth point of interest falls within the linear range of each system.
- To compare measurements between the pedestal and cuvette, a conversion factor is necessary and should be based on measurements made close to the target OD.

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