Technical Note

#8 OD600

Introduction

The growth of bacteria in liquid culture media is commonly monitored by measuring the optical density at 600 nm (OD600). OD600 measurements are typically used to determine the stage of growth of a bacterial culture, these measurements help ensure that cells are harvested at an optimum point that corresponds to an appropriate density of live cells. Growth of bacterial cells typically progresses through a series of consecutive phases including: lag, log, stationary and decline (Figure 1). In general, cells should be harvested towards the end of the log phase, using the optical density of the samples to determine when this point has been reached. Cells are routinely grown until the absorbance at 600 nm (known as OD600) reaches approximately 0.4 prior to induction or harvesting.

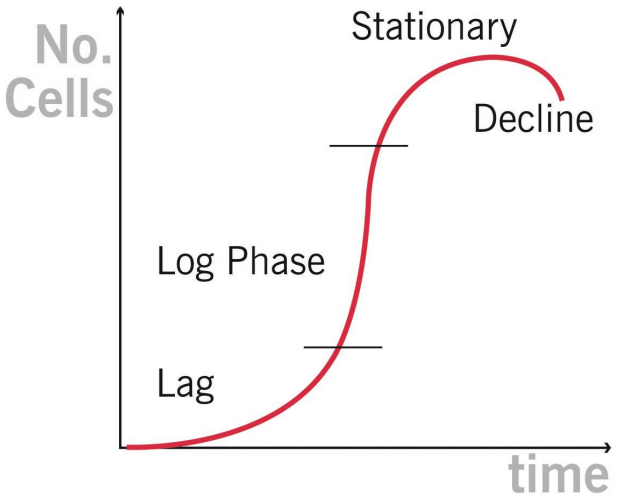

Figure 1: Bacterial growth curve

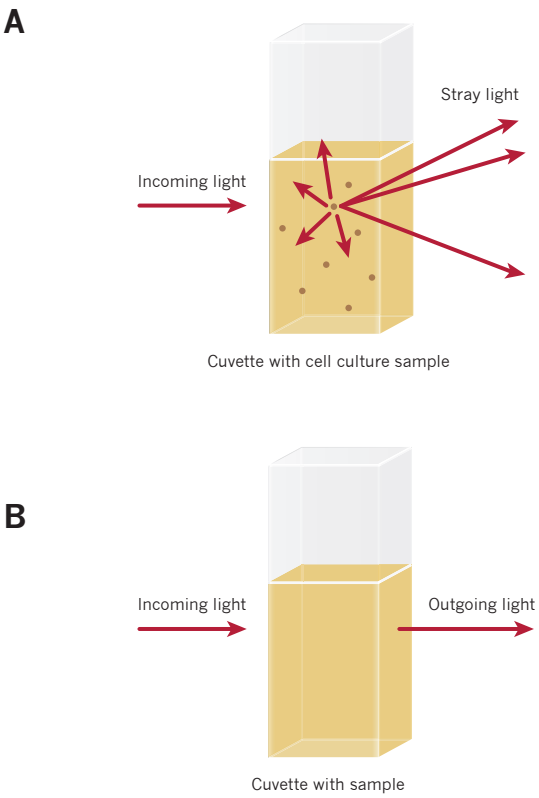

Figure 2: (A) **Light scattering of turbid sample.** The incoming light is emitted by the cells/particles in the sample randomly. This results in an outgoing stray light in undefined direction.
(B) **Light absorption of clear sample.** The incoming light is reduced by sample absorption (outgoing light) depending on sample concentration and wavelength. The Beer-Lambert law describes the correlation of incoming/outgoing light and sample concentration.

Optical density, in the case of OD600 measurements results from light scattering rather than light absorption (Figure 2).

The result of light scattering measurements (OD600) can vary between different photometer types depending on the optical setup.

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OD600 Measurements on different photometer types

For turbid samples such as cell cultures, the absorbance measured is due to light scattering, and not the result of molecular absorption. Because the extent of light scattering is affected by the optics of the system (distance between the cell holder and instrument exit slit, monochromator optics, slit geometry, etc.), different photometer types will tend to give different OD600 readings for the same turbid sample (Figure 3).

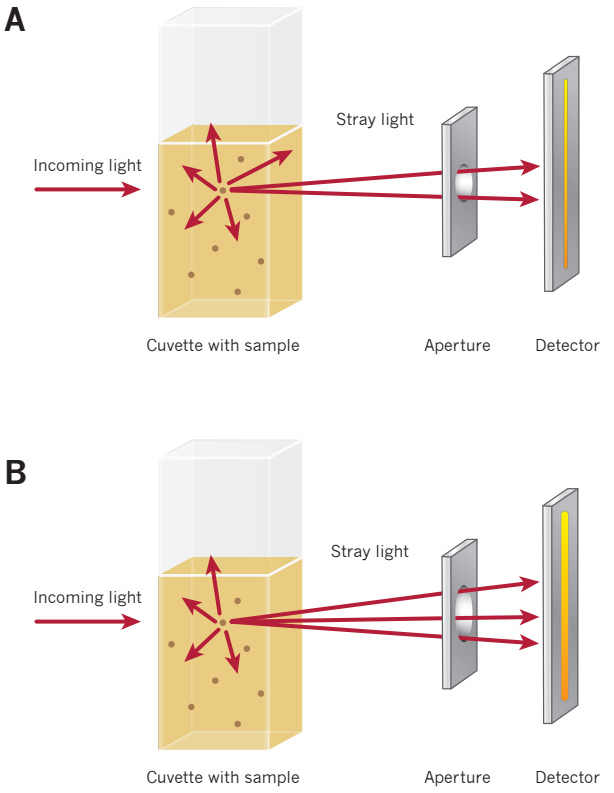

Figure 3: (A) Higher absorbance reading.
(B) Lower absorbance reading.

Results from different photometers cannot be compared directly for OD600 measurements. Consistently utilizing the same photometer for OD600 measurements guarantees the most reproducible results. Results from different photometer models must be normalized using appropriate calibration curves.

Normalization of OD600 measurements

The NanoPhotometer® comes with a correction factor of 1 as default. To compare OD600 values between different photometers, it is necessary to determine the constant deviation or ratio between the absorbance values for the same sample from each instrument and use this correction factor within the parameter setting “Correction” of your NPOS Software to normalize the results. A calibration curve can be constructed by comparing measured OD600 to expected OD600.

Cell/ml Calculation

The NPOS software is capable of calculating cells/mL in a sample. The default value used in the parameter settings of the NPOS software (Factor: 5, Multiplier: 100,000,000) is the factor commonly used for *E.coli* (1 OD600 = 5 x 10^8 cells/ml). Due to the fact that OD600 measurements are dependent upon the shape and size of the bacterial cells in a culture, the cells/mL value for cultures other than *E. coli* must be determined with an appropriate factor/ multiplier for the sample type.

Ratio values for commonly used cultures can be found in the literature. Alternatively, cells can be manually counted using a microscope and slide as an additional method to determine the number of cells equal to 1 OD600.

Cuvette vs. NanoVolume Measurements

OD600 measurements results from light scattering rather than light absorption. Therefore the use of 10 mm path length disposable cuvettes is recommended for optical density measurements of cell culture solutions. The amount of cells in a sample is reflected in the reading and the likelihood of fluctuating amount of cells in a drop from sample to sample can be considered as extremely significant. It is therefore recommended to utilize cuvettes for OD600 readings. The cuvette measurements provide a larger volume of sample thereby reducing the margin of error and generating more reproducible results.

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