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.

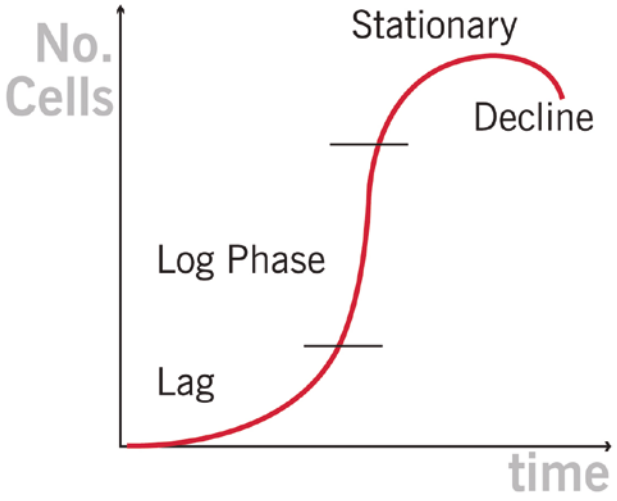

Figure 1: Bacterial growth curve

Optical density, in the case of OD600 measurements results from light scattering rather than light absorption. Different photometers therefore can produce varied result values. These variations stem from photometer models having distinctive optical setups.

OD600 Measurements

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. Therefore, if results from different photometers are to be compared, they must be normalized first using appropriate calibration curves.

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 factor within the parameter setting “Correction” of your NPOS Software. A calibration curve can be constructed by comparing measured OD600 to expected OD600.

Cell/ml Calculation

The NPOS software can calculate as a result a value for cells/ml. The default value in the parameter settings (Factor: 5 and Multiplier: 100,000,000) of the NPOS software is the one 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* needs to be determined.

Ratio values for commonly used cultures may be available 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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