OD_{600} Measurements

Technical Note 168

Introduction

The turbidity measurement of microbial cultures is a commonly used method to determine the growth phase or cell number in an actively growing culture. Most often, these determinations are done using a spectrophotometer to measure the absorbance at 600 nm. The measurements are however a measure of light scattering rather than a measurement of absorbed light This fact is important as OD_{600} values may differ amongst cell types using one instrument or when measuring the same cell sample on different instruments.

The purpose of this note is to highlight the source of the potential differences in OD_{600} measurements and present a method to normalize results when using the DS-11 spectrophotometer for cell density determinations.

Variation Source: Cell Types

For optical density measurements the light passing through the sample is scattered in random directions by particles in the sample. This light scattering is a function of both the specific cell size and shape as well as the density of the cell suspension. In addition dead cells and cell debris may contribute to light scattering. Different cell types at the same density (i.e cells per mL) may result in different OD_{600} values when measured on the same instrument.

Variation Source: Optical Configurations

It is well known that optical configurations of spectrophotometers play a role in the light scatter detected by a specific instrument. Different OD_{600} values will be reported for the same bacterial culture when measured on spectrophotometers with different optical set-ups.An OD_{600} of 0.8 using one instrument can be reported as 0.5 on another without being incorrect on either unit.

Variation Source: Cuvette vs Microvolume

DS-11+/DS-11 FX+ spectrophotometers enable the measurement of microbial cultures using either the 1 µL microvolume mode or various path length cuvette modes. The optical paths used for these two modes are different and therefore the reported OD_{600} values will differ for the same sample.

Conversion Factors

The DS-11 microvolume mode enables much higher OD_{600} values using its patented automatic path length adjustment. Keeping in mind that the microvolume and cuvette modes use different optical configurations, it may be useful to establish a conversion factor when comparing values measured using the two modes.

$$\text{Factor} = \frac{\text{Cuvette } OD_{600}}{\text{Microvolume } OD_{600}}$$

It is best to determine the factor using measurements close to the desired target OD_{600} value. Account for dilutions as appropriate. An equivalent factor may be determined and then applied to measurements using different spectrophotometers.

Empirical Target Values

Many investigators rely on target OD_{600} values obtained from literature sources when harvesting microbial cell cultures or determining the correct density to inoculate a culture for protein expression studies. Unfortunately, these target values may not be appropriate for the combination of the cell type and instrument in use. It is recommended that a growth curve be generated for each cell type when using a new spectrophotometer to ensure target values correlate with the desired growth phase of the culture.

Linear Range

Before generating growth curves, it is important to determine the linear range for each cell type for the specific measurement mode (cuvette or microvolume) to be used.

Cuvette based instruments generally have an upper OD limit of around 1.5 which may not be sufficient to cover the entire growth cycle of the culture. This upper limit may not be practical to use as some cultures do not exhibit linear responses at high OD_{600} values. In addition, cultures in the death phase may exhibit different light scattering behaviors. Therefore careful dilutions of the culture must be made in order to plot the entire growth curve or determine the limit or growth phases at which measurements no longer linearly correlate with cell density.

OD600 Values and Cell Number

The DS-11 + software allows the user to enter a cell number factor that will be multiplied by the measured OD_{600} value to calculate an approximate cells/ml concentration. As previously discussed, OD_{600} values are dependent on the size and shape of the cells the solution being sampled as well as the cell density. An OD_{600} value of 1 might equal approximately 1 $x10^8$ cells for one cell type yet equal only 0.5 $x10^8$ cells for another.

To use this feature, it is recommended that appropriate cell number conversion factors be initially determined for each cell type by constructing a calibration curve for OD_{600} values correlated to cell numbers by colony forming units grown on agar plates. Be sure to multiply by dilution factors when appropriate.

Yeast cells are generally larger than bacterial cells. In general an OD_{600} value of 1 will be equivalent to fewer yeast cells than for bacterial cells.

Best Practices

DS-11+ Cuvette Mode (recommended)

- Ensure the culture is well mixed and cells have not settled prior to taking an aliquot from the suspension.
- Use high quality plastic cuvettes or quartz cuvettes with a Z heights of 8.5 mm.
- Use clean cuvettes for each sample measurement. Clean cuvettes according to the manufacturer's recommended protocol..
- Ensure the cuvette is inserted in the proper orientation.

DS-11/DS-11+ Microvolume Mode

- Clean both sample measurement surfaces prior to making the Blank measurement.
- Ensure the culture is well mixed and cells have not settled prior to taking an aliquot from the suspension.
- Use 1 µL samples for routine measurements.
- Use a fresh aliquot for each measurement.
- Use a fresh tip to deliver each sample aliquot.
- Avoid introducing bubbles when pipetting samples onto the measurement surfaces.
- Use a dry lab wipe to remove the sample from both the top and bottom surface immediately after each measurement.

Conclusion

The DS-11 /DS-11+ spectrophotometer enables the measurement of both yeast and microbial cultures using the preconfigured OD_{600} app. To ensure the best results, target OD_{600} values should be empirically determined for each cell type for both the microvolume and cuvette modes.

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