Tech Note

SpecPlate

OD600 monitoring of microbial growth with SpecPlate

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OD600 measurements are a key method for tracking microbial growth by evaluating culture turbidity, providing insights into cell density over time. This Tech Note demonstrates how the use of SpecPlate enhances and streamlines this measurement method.

Introduction

Optical density at 600 nm (OD600) measurements are a widely employed technique for monitoring microbial growth during cultivation. This method provides a rapid means to estimate cell concentration, which is crucial for optimizing fermentation conditions. OD600 measurements rely on light scattering and absorbance phenomena when a beam of light passes through a sample; the more cells present in the sample, the more light is absorbed and scattered. Under specific conditions, the value of the attenuated light is linearly proportional to the amount of biomass, similar to the Lambert-Beer law. However, when the light path length or biomass concentration is too high, this linear relationship no longer holds. In such cases, the sample must be diluted, and the measurement result subsequently multiplied by the dilution factor. Each additional pipetting step is inherently prone to error; due to pipetting inaccuracies, the measurement result may deviate from the true value. Furthermore, since the exact concentration of a sample is mostly unknown beforehand to the experimenter, it is not always clear which dilution factor to choose. In such cases, an iterative approach may be necessary, which can be time-consuming.

The SpecPlate enables the measurement of up to 96 samples. Unlike typical microplates, the SpecPlate does not have the standard 96 circular wells but rather 96 multi-measurement structures (Fig. 1). The added sample is distributed through a channel system, and absorbance can then be determined at four points with different path lengths (100, 700, 1400, and 2000 µm). This design ensures that the absorbance determination remains within the linear range through the variation of pathlength. With this four-chamber system, the SpecPlate has an arrangement equivalent to a 384-well plate and can be read by standard microplate readers.

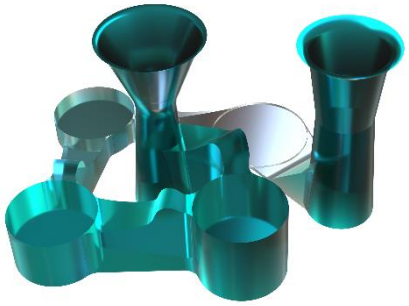


Figure 1 SpecPlate measurement structure

SpecPlate allows for accurate OD600 measurements without the need for manual dilution. Due to the various path lengths, the SpecPlate ensures that absorbance measurements remain within the linear region across a wide range of sample concentrations. This minimizes potential errors associated with dilution steps and pipetting. To facilitate an introduction to the SpecPlate, this Tech Note provides some guidance for implementing best practices for integrating the SpecPlate into microbial cultivation monitoring.

Material

- SpecPlate (PHABIOC, 400100)
- Standard 96-well microplates (µClear, Greiner Bio-One)
- Standard microplate reader (SpectraMax iD3, Molecular Devices)
- Manual pipette

Methods

An overnight shake flask cultivation of the bacterium *Sporosarcina pasteurii* was prepared. A dilution series of the culture was analyzed using a standard 96-well microplate and the SpecPlate.

Standard microplate

In a standard microtiter plate, samples must be diluted to fit within the linear range. If the OD600 of an unknown process is not estimated correctly, insufficient dilution may lead to absorbance outside the linear range, underestimating the OD600.

To illustrate this, 16 dilutions of the bacterial culture were prepared, ranging from 0% to 100% in steps of 5% for lower concentrations (0%, 5%, 10%, ..., 55%) and larger steps for higher concentrations (70%, 85%, 100%). These dilutions were further processed directly in a standard microplate, with each sample diluted 1:10 and 1:20 in separate wells, resulting in a final volume of 200 µL per well. The absorbance of all wells was then measured at a wavelength of 600 nm.

For the 1:20 dilution, the measured absorbance values showed a linear correlation with the corresponding dilution factors across the entire range. In contrast, the absorbance values for the 1:10 dilution exhibited high linearity up to an absorbance of 0.6. Beyond this threshold, the slope decreased due to nonlinear light attenuation.

This nonlinear light attenuation must be considered when interpreting absorbance results. If measurements fall outside the linear range but are still extrapolated using the dilution factor, the resulting OD600 values will be underestimated. In this example, selecting the incorrect dilution factor (1:10 instead of 1:20) led to a 19% underestimation of the OD600 at the highest concentration (100% bacterial culture, Fig. 2).

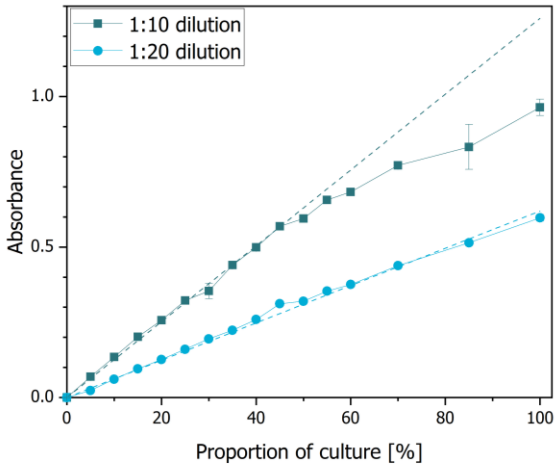


Figure 2 Absorbance of diluted S. pasteurii culture, measured with a standard 96-well microplate. The dashed lines indicate the linear fit at small absorbance values. The error bars depict the standard deviation (N = 3).

SpecPlate

With the SpecPlate, no dilution is needed. A single sample addition provides four values for different path lengths (Fig. 3), allowing the selection of the appropriate measurement result, which falls in the linear measurement range. In our example, even without any dilution and at high cell densities, at least one value (in this case at 100 µm) always falls in the linear measurement range. Consequently, there is no risk of choosing the wrong dilution factor. OD600 values are conventionally standardized to a 1 cm path length. However, a standard 96-well plate does not provide this path length. To convert measurements to a 1 cm path length, a

correlation must first be established by comparing readings from the plate to those obtained with a traditional 1 cm cuvette. While the SpecPlate features defined path lengths, an initial calibration using a cuvette is recommended to ensure correlation with the specific culture being measured.

The experiment showed a lower coefficient of variance in OD600 measurements with the SpecPlate (0.3% coefficient of variation at 2000 µm vs. 2.4% for a 1:10 dilution in a standard plate), due to the fixed path length of the SpecPlate compared to the slightly varying path length in open microplate wells due to pipetting inaccuracies. The elimination of the dilution steps also permits saving of time.

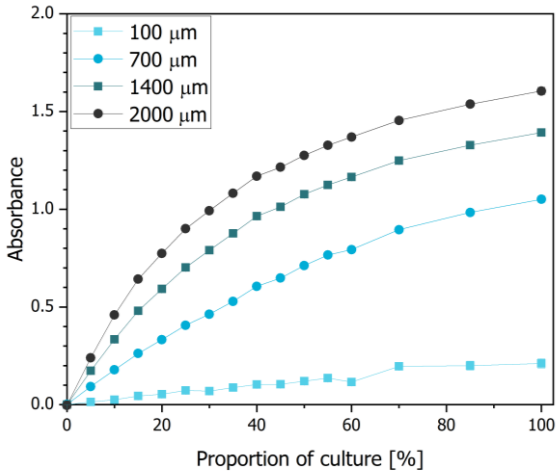
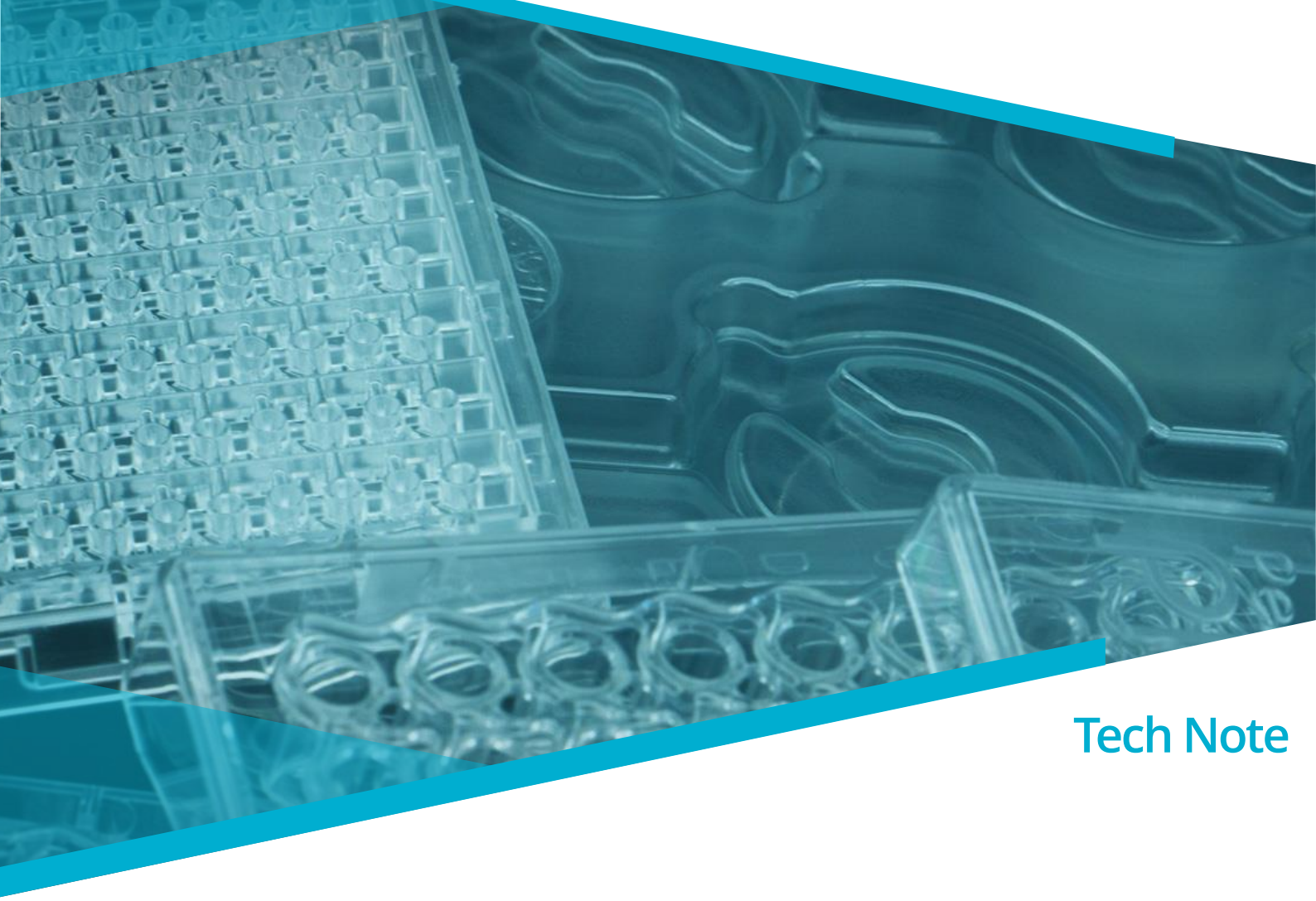


Figure 3 Absorbance of S. pasteurii culture, measured with the SpecPlate. The hardly visible error bars depict the standard deviation (N = 3).

Conclusion

In summary, the SpecPlate demonstrates clear advantages over conventional 96-well microplates, particularly in measurement accuracy and workflow efficiency. This increased workflow efficiency is especially important for automated sample analysis (see Technote "Best Practices for Manual and Automated Pipetting with SpecPlate"). The ability to achieve accurate OD600 readings without estimating and performing dilutions eliminates guesswork and reduces potential pipetting errors, leading to more reliable results. These benefits make the SpecPlate a practical and efficient tool for bioprocess development, simplifying microbial growth monitoring while improving overall data quality.

***Version 1**: Changes, including technical, reserved. 04.12.2024*



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