Determining equivalent onset optical density (OD) values on the Sievers* Eclipse BET platform

APPLICATION NOTE

Purpose

This application note demonstrates that the onset optical density (OD) values used in the Sievers Eclipse Bacterial Endotoxins Testing (BET) platform are equivalent to those used in 96-well microplate testing. As the Sievers Eclipse microplate has a different shape and path length, OD values need to be recalculated using Beer's Law and the equation for absorbance. Equivalency of these OD values is important because in kinetic chromogenic assays the onset OD value is used to determine onset times for standards and samples.

Overview

Kinetic endotoxin assays rely on an inverse relationship between the change in OD and endotoxin concentration. OD is the "measure of the amount of light absorbed by a suspension or a solution of an organic molecule at a specific wavelength, as measured by a spectrophotometer."[1] Software reports OD values over time as light passes through optical wells containing standard or sample mixed with Limulus Amoebocyte Lysate (LAL). For kinetic assays, the onset OD is a constant value used to determine onset or reaction times. A sample or standard with a high endotoxin concentration will react quickly and produce a yellow color, which results in less light reaching the photodiode(s) and a subsequent rise in the OD values. The shorter the onset time (i.e., the time it takes to reach the onset OD), the more endotoxin in the sample.

Upon completion of each assay, a standard curve is produced which shows the linear correlation between the log onset time and the log concentration of standard endotoxin. The onset times for reactive samples and positive product controls (PPCs) are then referenced against the standard curve to provide a quantitative endotoxin result. Onset OD values are optimized for different assays and are outlined in each manufacturer's instructions for use (IFUs). These values may consist of one set value or a range of values. Onset OD is also referred to as threshold OD or reaction OD.

Beer's Law

The OD values documented by manufacturers are determined using Beer's Law and the measured thickness of the microplate containing liquid (i.e., path length). Beer's Law, also known as Beer-Lambert's Law, states that "the concentration of a chemical solution is directly proportional to its absorption of light."[2]

Beer-Lambert's Law

$$A = \varepsilon bc$$

Where:

A = Absorbance (no units)
ε = Molar absorptivity (L mol^{-1} cm^{-1})
b = Length of solution the light passes through (cm)
c = Concentration of compound in sample (mol L^{-1})

To fully understand the equation, it is important to recall the equation for absorbance. Absorbance is determined using light intensity. Intensity is measured across a reference cell or one without a solution (I_o) and then a sample cell or one with solution (I). Using these two values, absorbance can be calculated using the following equation.

Absorbance

$$A = log_{10}\left(\frac{I_o}{I}\right)$$

The shape and size of the well is important when considering absorbance values. A solution in a cube shaped well with a path length of 1 cm will have lower levels of absorbance than the same solution in a tube-like well with a path length of 100 cm because there are

less molecules for the light to interact with. The wells on the Sievers Eclipse microplate have a different shape and path length than the wells on the

96-well microplate, which is why the onset OD values need to be recalculated using the two equations referenced above.

OD Equivalency with Sievers Eclipse

The path length of a 96-well microplate varies slightly depending on the manufacturer or vendor. This is because the well on a flat bottom 96-well microplate is often a truncated cone, made for easy release from the molding. Using the values obtained from several large-scale plate manufacturers, instrument manufacturers, and LAL vendors, the path length was determined based on 200 µL of solution in the well, which led to a range of values from 5.707 mm – 6.306 mm.

In order to narrow this range down to one value, measurements were completed again on a variety of 96-well microplates in the Veolia Analytical Instruments applications lab in Boulder, CO. This led to a calculated path length value in the middle of the range determined from the various sources. This value was then used in the equation below to convert onset OD values used in 96-well plate testing into an equivalent value that is suitable for the Eclipse platform.

$$OD_{Eclipse} = OD_{\text{96 well}} \, x \, \left(\frac{Path\ Length_{\ Eclipse}}{Path\ Length_{\text{96-Well}}}\right)$$

Using the calculation above with the path length values determined for the 96-well microplate and Eclipse microplate, the optical density values in **Table 1** were calculated for use in the Eclipse software.

Table 1: Reference Onset Optical Density Values**

96-well plate onset optical density value (OD units)	Sievers Eclipse onset optical density value (OD units)
0.03	0.01296
0.05	0.0216
0.08	0.03456
0.10	0.0432
0.15	0.0648
0.20	0.0864

**For use on the Sievers Eclipse platform.

These OD values were tested side-by-side on the 96-well microplate and Eclipse microplate to ensure all endotoxin, PPC, and %CV (coefficient of variation) values proved to be equivalent or better. If a range of OD values was provided by the lysate manufacturer, the entire range was recalculated and tested on the Sievers Eclipse platform. Equivalency was confirmed across all ranges, proving the conversion was performed correctly and the data generated were accurate.

If various onset OD values are used in your lab, it is recommended to properly test and determine which value is optimal for each sample, as the onset OD value cannot be changed in the Sievers Eclipse software after the assay completes. **Table 1** may be used to help determine equivalent onset OD values to what you currently use for your samples.

Conclusion

While the onset optical density values are different than the 96-well microplate, equivalent values for the Eclipse BET platform have been accurately calculated through well-established physical equations. Therefore, users can be confident in the endotoxin results measured with the Eclipse platform. For more guidance, please contact your local Sievers representative or applications specialist.

References

1. McCullough, Karen Zink. The Bacterial Endotoxins Test: A Practical Approach. DHI Publishing, LLC, 2011.
2. Helmenstine, Anne Marie. Beer's Law Definition and Equation. ThoughtCo, 2019. https://www.thoughtco.com/beers-law-definition-and-equation-608172

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