Technical Overview

Dual-Wavelength Measurements Compensate for Optical Interference

An experiment with plate lids and induced condensation using the Agilent BioTek ELx808IU absorbance reader

Abstract

Dual wavelength is used in many microplate-based applications to reduce optical interference caused by scratches, fingerprints, or other matter that absorb light equally at both wavelengths. For example, many investigators prefer to read microplate-based assays with lids or membrane seals in place to reduce biohazards, as well as evaporation. As a result of using lids, condensation may collect on the lid during the assay process. To illustrate the benefits of dual wavelength in these types of applications, experiments were performed showing what effect on results a plate lid, with or without condensation, can have when readings are taken at single versus dual wavelength. The experiments demonstrated that when the plate is read at two wavelengths and the difference in optical densities is computed, this technique adequately compensated for these effects.

Experimental results

The experiment performed compared single and dual wavelength measurement techniques using an Agilent BioTek ELx808IU absorbance reader and a filled microplate with and without lid and condensation present. A NUNC MaxiSorp 12-1x8 removable strip microplate was used. Two different plate covers were tested: a NUNC 96-well hard cover, and a generic adhesive cover common in many assay kits on the market. An 8-channel micropipette was used to dispense 200 µL of deionized water into wells A1 through A12, and a 3:5 dilution of Agilent BioTek QC Dye #2 and QC Dye #3 (part numbers 7120783 and 7120784, respectively) into the remaining wells. Plates were then read at single and dual wavelength. Plate lids were applied to the plates and they were read again at single and dual wavelength. This was followed by a 30-minute incubation at 5 °C to induce condensation, and the plates were remeasured at single and dual wavelength with visible condensation on the plate lid. Table 1 shows a chart of the experimental process including the mean plate optical density (OD) and mean plate CV% for each reading.

The presence of an adhesive plate cover at single wavelength adds just over 10% to the mean OD (P1, R1 versus P1, R3). At dual wavelength, however, the difference in mean OD with and without the adhesive plate cover is only 1.5% (P1, R2 versus P1, R4). Following the 5 °C incubation used to induce condensation, the results are even more noticeable. At single wavelength, there is an almost 25% increase in mean OD (P1, R3 versus P1, R5) caused by the presence of condensation on the plate lid, while the dual wavelength mean OD compensates as shown by a less than a 0.5% increase (P1, R4 versus P1, R6).

The results for the NUNC cover are similar, but less pronounced. Using single wavelength measurements, it adds just over 3.7% to the mean OD (P2, R1 versus P2, R3), while with dual wavelength the difference is almost undetectable at 0.02% (P2, R2 versus P2, R4). Following the 5 °C incubation, the compensation of dual wavelength clearly makes a difference. At single wavelength there is an almost 20% increase in mean OD (P2, R3 versus P2, R5) caused by the presence of condensation on the plate lid, while the dual wavelength mean OD compensates as shown by a less than 0.046% increase (P2, R4 versus P2, R6). The change in CV% for the NUNC cover also illustrates the compensatory properties of dual wavelength. At single wavelength, there is over a 100% change in CV% with and without condensation (P2, R3 + P2, R5), but only approximately a 4% change when condensation is present at dual wavelength (P2, R4 + P2, R6).

Table 1. Plate readings with mean plate ODs and mean plate CV% for comparison of single- and dual-wavelength techniques.

Plate, Read	Wavelength	Lid Present	Visible Condensation	Mean Plate OD	Mean Plate CV%
Adhesive Soft Plate Cover					
P1, R1	Single (450)	No	No	1.107	0.646
P1, R2	Dual (450, 630)	No	No	1.07	0.501
P1, R3	Single (450)	Yes	No	1.229	0.721
P1, R4	Dual (450, 630)	Yes	No	1.085	0.604
Refrigeration					
P1, R5	Single (450)	Yes	Yes	1.566	0.670
P1, R6	Dual (450, 630)	Yes	Yes	1.086	0.730
NUNC Hard Plate Cover					
P2, R1	Single (450)	No	No	1.109	0.726
P2, R2	Dual (450, 630)	No	No	1.071	0.734
P2, R3	Single (450)	Yes	No	1.15	0.669
P2, R4	Dual (450, 630)	Yes	No	1.069	0.725
Refrigeration					
P2, R5	Single (450)	Yes	Yes	1.37	1.640
P2, R6	Dual (450, 630)	Yes	Yes	1.064	0.697

Conclusion

Condensation on plate lids can be significant whenever a temperature differential exists between the plate and the fluid at the time of pipetting, or whenever a filled plate experiences an appreciable change in temperature. If condensation does form on the plate lid, light scattering effects can significantly change absorbance readings and CVs. These detrimental effects of condensation are compensated by taking advantage of readings at two wavelengths, and subtracting absorbance at the reference wavelength from those at the reading wavelength.

It is strongly advisable for any investigator contemplating performing assay readings with plate lids in place to acquire a microplate reader that permits dual-wavelength readings over a broad light spectrum and that physically accommodates the covered plate. These criteria are met by the Agilent BioTek ELx808IU and ELx800UV absorbance readers, which feature a wavelength range of 340 to 900 nm and 340 to 750 nm, respectively, while also accommodating a variety of plate lids within the reading chamber.

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