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Application Note

Dual-Wavelength Measurements Compensate for Optical Interference

An Experiment with Plate Lids and Induced Condensation using the BioTek ELx808IU Absorbance Reader

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, Bio-Tek performed experiments showing what effect on results a plate lid, with or without condensation, can have when readings are taken at single vs. 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 a Bio-Tek ELx808IU 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 200µl of deionized water into wells A1-A12, and a 3:5 dilution of QC Dye #2 and QC Dye #3 (P/Ns 7120783 and 7120784 respectively, Bio-Tek Instruments, Winooski, VT) into 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^{o}C to induce condensation, and the plates were re-measured 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.

BioTek Instruments, Inc., P.O. Box 998, Highland Park, Winooski, Vermont 05404-0998 USA
 TEL: 888-451-5171 FAX: 802-655-7941 Outside the USA: 802-655-4740 E-mail: customercare@biotek.com
www.biotek.com

Adhesive soft plate cover

Plate, Read	Wavelength	Lid Present	Visible Condensation	Mean Plate OD	Mean Plate CV%
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

Plate, Read	Wavelength	Lid Present	Visible Condensation	Mean plate OD	Mean Plate CV%
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

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

The presence of an adhesive plate cover at single wavelength adds just over 10% to the mean OD (P1,R1 vs. P1,R3). At dual wavelength, however, the difference in mean OD with and without the adhesive plate cover is only 1.5% (P1,R2 vs. P1,R4). Following the 5^{o}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 vs. 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 vs. 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 vs. P2,R3), while with dual wavelength the difference is almost non detectable at 0.02% (P2,R2 vs. P2,R4). Following the 5^{o}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 vs. 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 vs. 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 (P2R3 + P2R5), but only about a 4% change when condensation is present at dual wavelength (P2R4 + P2R6).

Conclusions

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 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 Bio-Tek® ELx808IU and ELx800UV readers, which feature a wavelength range of 340 to 900nm and 340 to 750nm respectively, while also accommodating a variety of plate lids within the reading chamber.

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