MOLECULAR DEVICES

TECHNICAL NOTE

Effect of microplate materials on UV absorbance measurements

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Microplates used for absorbance measurements are commonly made of polystyrene, cyclic olefin copolymer (COC), or quartz. All of these materials work well for making measurements in the visible wavelength range (400–700 nm), but in the ultraviolet (UV) range (<400 nm) some materials absorb light and are unsuitable for applications such as direct quantification of DNA, RNA, or protein, which are based on absorbance measurements made at 260 or 280 nm. The choice of a suitable microplate material for UV absorbance measurements is critical, as transmission properties vary between materials. Standard polystyrene used in many microplates absorbs light in the UV range, resulting in high background. To minimize background in the UV range, quartz, COC, or microplates made with 'UV-transparent' plastics such as fluorocarbon (FC) are often used.

In this technical note, the SpectraMax® ABS Plus Microplate Reader was used to assess the UV absorbance of several microplates where the base of the wells (surface through which light passes during measurement on a microplate reader) consisted of polystyrene, COC, or FC. The SpectraDrop™ Micro-Volume Microplate, which consists of a holder containing two quartz slides between which 2- or 4-µL samples are positioned for measurement, was included for comparison. A microplate lid and a heat seal were also tested for their UV absorbance.

Materials

Instruments

- SpectraMax ABS Plus Microplate Reader (Molecular Devices cat. #ABS Plus)
- 4s3™ Semi-Automatic Sheet Heat Sealer (Brooks Life Sciences cat. #4TI-0655)

Microplates

- SpectraDrop Micro-Volume Microplate (Molecular Devices cat. #0200-6263)
- UltraVision™ Plate 384 (Brooks Life Sciences cat. #4TI-0214)
- UltraVision Plate 96 (Brooks Life Sciences cat. #4TI-0234)
- Vision Plate 384 (Brooks Life Sciences cat. #4TI-0204)
- Vision Plate 96 (Brooks Life Sciences cat. #4TI-0224)
- µClear UV-Star (Greiner Bio-One cat. #655801)
- µClear UV-Star (Greiner Bio-One cat. #788876)
- µClear CELLSTAR (Greiner Bio-One cat. #655090)
- Nunclon Delta Surface (ThermoFisher Scientific cat. #167008)

Microplate covers

- Universal Microplate Lid (Brooks Life Sciences cat. #4TI-0290)
- Clear Heat Seal (Brooks Life Sciences cat. #4TI-0541)

Name	Material	Base	Wall color	No. of wells
SpectraDrop Micro-Volume Microplate	Quartz	Clear	N/A	24 or 64
UltraVision Plate 384	COC	Ultra-clear	Black	384
UltraVision Plate 96	FC	Ultra-clear	Clear	96
Vision Plate 384	PS	190 µm clear	Black	384
Vision Plate 96	PS	190 µm clear	Black	96
µClear UV-Star	COC	135 µm clear	Clear	96
µClear UV-Star	COC	135 µm clear	Black	384
µClear CELLSTAR	PS	190 µm clear	Black	96
Nunclon Delta Surface	PS	Clear	Clear	96
Universal Microplate Lid	PS	Clear	N/A	N/A
Clear Heat Seal	Polyester	Clear	N/A	N/A

Table 1. Features of tested microplates and plate covers. The SpectraDrop Micro-Volume Microplate consists of a holder containing two quartz slides with 24 or 64 sample positions that can hold 2 or 4 µL each.

Methods

To test the optical clarity of the various microplates at low wavelengths, one well of each plate was filled with 100 µL (96-well) or 20 µL (384-well) distilled water, and its absorbance was measured from 200 nm to 400 nm in 1-nm increments. All wavelength scans were performed on the SpectraMax ABS Plus Microplate Reader, which has a bandwidth of 2 nm, using SoftMax® Pro Software. To test the effect of plate covers, the UltraVision™ 96-well microplate with clear walls and ultra-clear base was measured (a) without a cover, (b) with the clear Universal Microplate Lid, and (b) sealed with a Clear Heat Seal using the 4s3™ Semi-Automatic Sheet Heat Sealer according to the manufacturer's instructions.

Results

The results of the spectral scans are shown in Figure 1 and Table 2. All plate materials tested have low absorbance in the visible range above 340 nm as expected, but major differences are evident for absorbance in the low (200 to 280 nm) and high (280 to 340 nm) UV ranges.

In the low UV range, best performance was demonstrated with quartz, FC- and COC-based microplate materials, with major differences observed in the range of 200 to 230 nm. In the zoomed diagram of the spectral scans from 200 to 240 nm, the differences in absorbance are apparent (Fig. 1, B). The SpectraDrop Micro-Volume Microplate, with quartz slides, had the lowest background absorbance of 0.2 OD at 200 nm. The clear UltraVision FC 96-well microplate (FC_UltraVision (clear, 96)) showed the best performance of the tested microplates, with absorbance of 0.3 OD at 200 nm. Both COC microplates, the µCLEAR UV STAR 96- and 384-well plates, had background absorbance of 1.2 OD at 200 nm; this decreased to 0.1 OD at 230 nm. The third tested COC-based plate, the UltraVision 384-well microplate, however, absorbed at 2 OD at 200 nm, decreasing to 0.2 OD at 230 nm.

All other tested plates and covers proved to be incompatible with low UV-range assays, as they absorbed at OD values above 2.5 at wavelengths below 280 nm. These plates and covers may be appropriate for use with wavelengths above 280 nm.

Spectral scans of the µCLEAR CELLSTAR cell culture microplates, as well as the black-walled, clear-bottomed polystyrene Vision Plates show absorbance of 3.6 OD at 200 nm that decreases sharply at 280 nm and reaches a minimum at 320 nm (Fig. 1, A). The second tested cell culture microplate, the Nunclon Delta Surface plate, has a higher absorbance of 3.8 OD at 200 nm, decreasing to a minimum at 310 nm. Assays that are measured at 340 nm, including lactate dehydrogenase (LDH) assays, nicotinamide adenine dinucleotide (NAD/NADH) assays, and endotoxin assays using Limulus amebocyte lysate (LAL) can be accurately measured using these plates.

For some assays it is necessary to cover the wells of the microplate in order to avoid well-to-well contamination or evaporation. Different types of plate covers are available; here we tested a polyester heat seal and a polystyrene universal lid. As shown in Table 2, both of these absorbed from 3.6 to 4 OD in the range of 200 to 260 nm. Overall the polyester heat seal has lower absorbance at wavelengths above 260 nm, with both the heat seal and the lid showing much lower absorbance above 320 nm. Both plate covers are likely suitable for use with assays read above 320 nm.

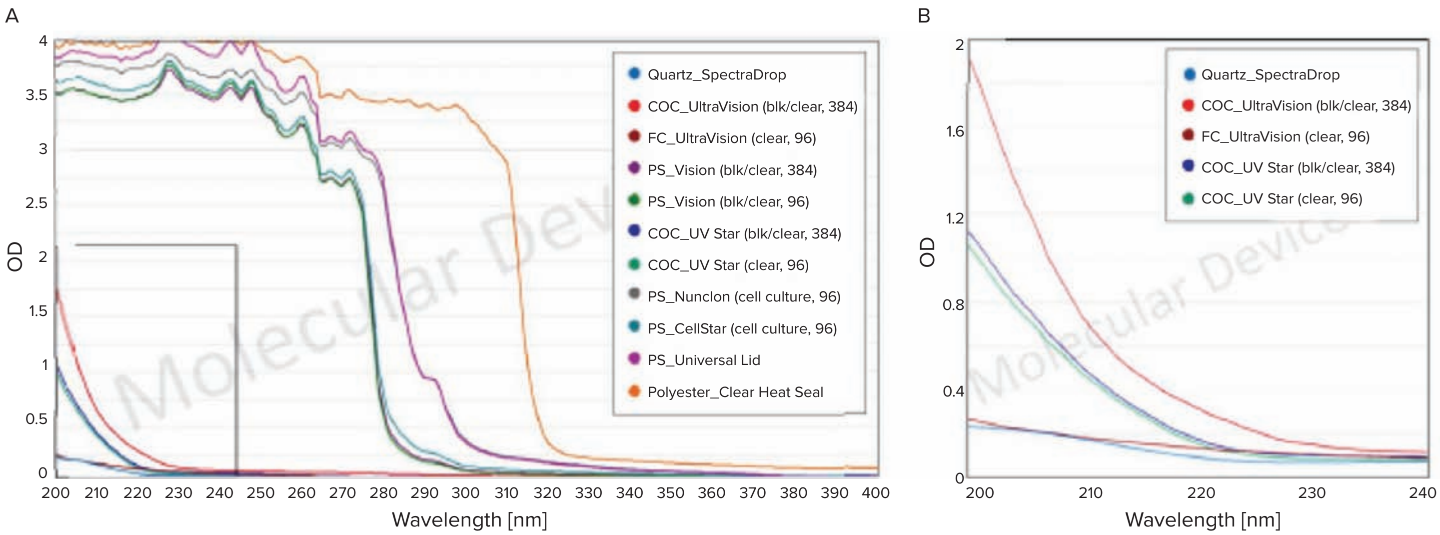

Figure 1. Absorbance profiles of different microplate materials. A: Spectral scan of different plates from 200 to 400 nm. B: Enlarged inset from A, showing absorbance from 200 to 240 nm for the five plates with the lowest absorbance.

	Optical density						
Legend	**200 nm**	**230 nm**	**260 nm**	**280 nm**	**300 nm**	**320 nm**	**340 nm**
• Quartz_SpectraDrop	0.234	0.069	0.044	0.039	0.038	0.036	0.035
• COC_UltraVision (blk/clr, 384)	1.928	0.158	0.110	0.092	0.079	0.072	0.066
• FC_UltraVision (clr, 96)	0.298	0.112	0.071	0.057	0.048	0.041	0.036
• PS_Vision (blk/clr, 384)	3.562	3.685	3.209	0.665	0.108	0.070	0.058
• PS_Vision (blk/clr, 96)	3.563	3.724	3.249	0.611	0.102	0.068	0.058
• COC_UV Star (clr, 96)	1.156	0.077	0.056	0.044	0.039	0.036	0.034
• COC_UV Star (blk/clr, 384)	1.143	0.092	0.062	0.049	0.043	0.039	0.036
• PS_Nunclon (cell culture, 96)	3.809	3.843	3.519	2.617	0.371	0.180	0.117
• PS_Cellstar (cell culture, 96)	3.679	3.762	3.294	0.797	0.148	0.099	0.117
• PS_Universal Lid	3.851	4.000	3.649	2.733	0.365	0.182	0.115
• Polyester_Clear Heat Seal	4.000	4.000	4.000	3.581	3.468	0.407	0.239

Table 2. Optical destiny of different plate materials at selected wavelengths. Absorbance values at the wavelengths indicated were taken from spectral scans performed on the different plates/covers.

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Conclusion

The comparison of microplates and plate covers shown here highlights the effect of different materials on absorbance in the UV range. For some materials, background absorbance can be significant. Therefore, when determining which microplate/cover to use for a given assay at a particular wavelength or range of wavelengths, it is important to determine absorbance of blank wells (e.g. buffer or water only) at each wavelength to assess the suitability of the microplate being considered. For example, a microplate used in the direct quantification of DNA and RNA, which are measured at 260 nm and 280 nm, should be tested to ensure that it absorbs minimally at these wavelengths.

In general, the manufacturers guidelines for each microplate should be observed, e.g. Greiner indicates an optimal range for the µCLEAR UV STAR down to 230 nm, although it was observed here that the absorbance of the microplate is minimal down to 220 nm.

Using regular polystyrene microplates can negatively impact results for assays measured at UV wavelengths. In this study, the quartz glass of the SpectraDrop slides (enabling measurement of 2- and 4-µL samples) showed the lowest background absorbance in the UV range, compared to other materials tested. For measurements of more typical assay volumes in the UV range, FC or COC microplates offer a lower-cost alternative to quartz, with low absorbance values at wavelengths commonly used in a variety of assays.

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