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Tech Note

# The Importance of Using the Appropriate Microplate for Absorbance Measurements in the Ultraviolet Region of the Spectrum

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*Does your microplate have the right stuff? The choice of 96-well microplate is critical when making absorbance determinations in the ultraviolet (UV) region of the light spectrum. In this report, we compare several different microplates for their background absorbance characteristics in the UV range.*

## Introduction

Because the absorbance of light by polystyrene or polypropylene in the visible portion of the electromagnetic spectrum is very low, historically there has been little concern for the background absorbance generated by the microplate itself when performing microplate based assays. If there was a concern, the usual method for correction was the utilization of dual wavelength measurements to correct for background. This method of correction is based on the premise that the background absorption of the microplate is consistent at the two different wavelengths used. However, this is not the case with most microplates in the UV range of the spectrum, making the choice of plates critical when measurements are taken. Historically, disposable polystyrene or polypropylene plastics and glass tend to absorb light in the UV range, necessitating the use of quartz wells when making absorbance measurements in the UV range. With the advent of “UV transparent” plastics, disposable microplates are now available for use in microplate readers capable of reading in the ultraviolet range.

## Materials and Methods

Individual wells were filled with 100 µl of distilled water and the absorbance was determined from 200 nm to 800 nm in 1 nm increments. Several different 96-well microplates were examined, including Hellma quartz (cat. # 730.009-QS), Costar UV plate (cat. # 3635), Polyfiltronics UV-MAX plate (cat. # UV-MAX), Nunc Maxisorp (cat. # 442404), and Costar polypropylene (cat. #3794).

All scanning absorbance determinations were made using a PowerWave™ 200 scanning microplate spectrophotometer (BioTek Instruments, Winooski, VT) in conjunction with KC4 data reduction software (BioTek Instruments, Winooski, VT) running on an external PC to control reader function and data capture.

## Results

As demonstrated in Figure 1, quartz microplates have the lowest background absorbance of the microplates tested. The Hellma 96-well microplates (A) have a background absorbance at 200 nm of 0.086. This value drops gradually to an average of 0.028, which varies little over the visible range of the spectrum up to 800 nm. Conventional polystyrene, such as the Nunc Maxisorp (E), or polypropylene microplates, such as the Costar polypropylene (D), exhibit absorbance values of approximately 2.800, which represents the maximal absorbance value at that wavelength for the reader we used in this study. The polystyrene plate is virtually opaque to light of wavelengths 200 nm to 275 nm, with the background absorbance dropping quickly with wavelengths above 275 nm to a value of 0.400 at 300 nm and less than 0.050 by 375 nm. Polypropylene, while equally opaque at 200 nm shows slightly less absorbance than polystyrene in the lower UV wavelengths. Polypropylene decreases rapidly from approximately 3.000 at 230 nm to less than 1.000 at 240nm, with a slight secondary peak from 250nm to 290 nm.

When wavelengths from 300 nm to 800 nm were examined the polypropylene microplate's background gradually decreases from 0.350 to 0.075.

The disposable "UV transparent" microplates demonstrate much better absorbance characteristics in the UV wavelengths than conventional plastic microplates. Although the Costar UV plate (B) has a background absorbance of approximately 1.800 at 200 nm, it quickly decreases to 0.100 by 230 nm and is 0.050 by 260 nm. The background absorbance in the visible light wavelengths was found to be approximately 0.025. The Polyfiltronics disposable UV microplate (C) has an absorbance at 200 nm of 2.500, which quickly drops to 0.090 by 250 nm. Interestingly, a small secondary peak was observed from 255 nm to 290 nm, where the background absorbance increased to 0.200. With wavelengths above 300 nm the background absorbance was very similar to that observed with the Costar UV microplate.

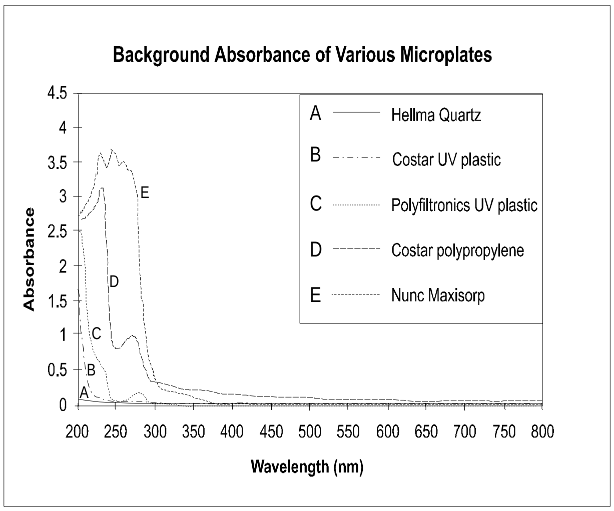


**Figure 1. Spectral analysis of several different microplates.** The indicated microplates were scanned form 200 nm to 800 nm in 1 nm increments using a PowerWave™ 200 scanning microplate spectrophotometer. In each case scans were performed on wells containing 100 µl of distilled water.

## Discussion

It is quite apparent that the choice of microplates is more complex when measurements are to be made in the ultraviolet wavelengths than in the visible portion of the spectrum. Because disposable microplates are generally made from some type of plastic polymer, background absorbance can be significant depending on the wavelength. In this study quartz was demonstrated to be superior in regards to its absorption characteristics to all of the disposable microplates tested. Quartz microplates, however, are very expensive and not disposable. Loss of a quartz plate due to breakage or contamination results in a significant monetary loss. Conventional polystyrene or polypropylene microplates, while quite adequate for absorption determinations in the visible light portion of the spectrum, are unsuitable for determinations in the UV range.

Disposable microplates specifically designed for use in the ultraviolet wavelengths are suitable for most applications in the UV range, but background absorbance varies depending on the wavelength and becomes significant if wavelengths lower than specified by the manufacturer are used.

Although we have utilized some of these microplates in wavelengths outside their specified range, we feel that it is important to note the consequence of doing so. If stable, superior (i.e., low) background absorbance at multiple UV wavelengths is absolutely necessary, then a quartz microplate is the solution. If, on the other hand, a disposable microplate is needed for UV absorbance determinations, then a specifically designed "UV transparent" microplate is necessary. If relationships between multiple UV wavelengths are to be examined, such as the $A_{260}/A_{280}$ ratio with nucleic acid samples (1), then appropriate blanking at each wavelength must take place in order to account for the differences in background absorbance of the microplate at different wavelengths.

The choice of microplate has to include several different factors. While quartz demonstrates superior characteristics in regards to background absorbance, it is not disposable, expensive, and can react with certain substances (e.g., hydrofluoric acid). The conventional polystyrene and polypropylene microplates are dependable, inexpensive, and offer adsorption characteristics that make them amenable to many different assays. However, because of their relatively high background absorbance, these plates are unacceptable for use in determinations that require measurements in the ultraviolet portion of the spectrum. Newly available "UV transparent" microplates offer many of the advantages of conventional disposable microplates along with relative transparency to ultraviolet light. Their background absorbance does vary to some extent with different wavelengths in the UV range so care must be taken to correct for this.

## References


(1) Held, P. "Nucleic Acid Purity Assessment Using $A_{260}/A_{280}$ Ratios". Application Note, BioTek Instruments, Winooski, VT.