Tech Note

Confidential

Qualifying Absorbance Readers in the UV

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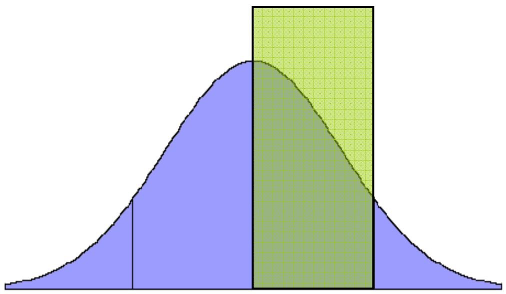
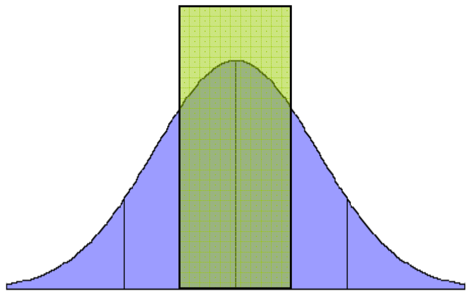
Many of our customers perform absorbance experiments in the UV range of the spectrum and are looking for ways to verify the performance of their instruments in that range. This article explains some methods for testing the performance of our instruments at lower wavelengths.

Method 1: The Absorbance Test Plate (p/n 7260522)

The neutral density filters used for the **intensity certifications** have a relatively constant OD over a large wavelength range, but have a rapid decrease in light transmission below 400 nm, and virtually no transmission below about 300 nm. These are used for OD intensity certifications in the visible range because even if the selected wavelength is slightly different, the readings will not change significantly. For filter-based readers, if the filters are slightly different from lot to lot with regard to the middle wavelength (as they are) the measurements will not be significantly affected. Similarly, with the monochromator-based readers, small shifts in wavelength will not significantly change the OD values. This is a NIST- verified method.

The Holmium Oxide filter is used for **monochromator certifications** (or wavelength certifications) to verify that when a specific wavelength is selected, the monochromator is truly at that wavelength. There are several Holmium Oxide peaks in the UV (at about 241, 279, 288, 334 and 360 nm) but **these cannot be used for intensity certifications**. Because the Holmium Oxide peaks are relatively sharp, if the monochromator middle wavelength (or filter middle-wavelength) and/or spectral bandpass vary you can get substantially different OD readings because different parts of the peak may be sampled. In the illustration below, the same filter bandpass (light green box) would give a lower OD reading in the example on the left-hand side than that on the right-hand side. It is not a NIST-verified method to use these peaks for intensity certification.





Method 2: The "Yellow Dye Test" for linearity at 260 nm for Monochromator-based readers (http://mktg.biotek.com/tektalk/2009/0309/TekTalk_0309.htm)

BioTek recently published a test that used dilutions of QC2 yellow dye solution (p/n 7120783) measured at 260 nm. This test is useful to determine whether an instrument gives a linear response at 260 nm. If the R^2 is 0.99 or higher, to 2.0 OD in a 96-well UV-compatible plate read in Normal mode, it passes the linearity specification of the monochromator-based readers. It cannot be used to certify OD intensity readings.

Method 3: Absorbance Liquid test 3 (ß-NADH solutions measured at 340 nm)

This test is outlined in the Qualification section of the user's Operator's Manual. It involves dilutions of ß-NADH solution measured at 340 nm. It is read 5 times and the linearity and repeatability are calculated. It is a validated method with instrument specific specifications for repeatability and linearity. It cannot be used to certify OD readings. It can be used with all of our absorbance readers (both filter and monochromator-based).

Conclusions:

The approach to verifying an absorbance reader's performance in the UV wavelength depends on the instrument and user's requirements. The Absorbance Test Plate's holmium oxide filter can be used to verify wavelength accuracy in the UV range for monochromator-based readers, as described in Method 1 above. Method 2 describes a method for checking the linearity of monochromator-based readers at 260 nm, and Method 3 outlines a validated procedure to verify an absorbance reader's repeatability and linearity at 340 nm specifically.

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