Bauer Core Standard Protocol		
Title: Using the Spectramax Plus 384 UV-Visible Plate Reader		
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1. Purpose

This protocol provides instructions for using the Spectramax Plus 384 UV-Visible Plate Reader to read absorbance between 190 – 1000 nm. The protocol is designed as a reference and is not a substitute for training. Users must complete a training session before using any of the Bauer Core's instrumentation.

2. Materials

2.1. UV Transparent Plates (e.g. Corning plate #3635) are needed at wavelengths below 340nm. Above 340nm, any 96 or 384 well plate can be used.
2.2. Samples (minimum 100µl volume)

3. Instrumentation

Molecular Dynamics Spectramax Plus 384 UV-Visible Plate Reader

4. Reagent preparation

none

5. Procedure

5.1. Open the SoftMax Pro software using the Spf shortcut on the desktop.
5.2. Click on the Instrument Icon.
    5.2.1. Choose COM 4 and Spectramax Plus 384.
5.3. Click on Plate 1 – Setup.
    5.3.1. Assay type.
        5.3.1.1. Endpoint: most common assay, 1 scan/well, up to 4 wavelengths. Use autocutoff to ensure excitation wavelength isn't detected.
        5.3.1.2. Kinetic: will scan several times over selected run time. Choose run time and interval.
        5.3.1.3. Spectrum: will scan over a range of wavelengths.
    5.3.2. Wavelengths – choose the wavelength(s) you would like to read.
    5.3.3. Pathcheck: Used to normalize the results to a 1cm pathlength.
        5.3.3.1. Choose the "water constant" to use a pre-programmed constant.
        5.3.3.2. Choose cuvette reference determine the constant from a water sample in the cuvette holder.
        5.3.3.3. Background constant: If you don't want to run a pre-read blank

enter your pre-determined background constant for each wavelength.

5.3.4. Automix and Blanking:

5.3.3.1. Automix will shake the plate for 5s (not very powerful).

5.3.3.2. Use "pre-read" to read a blank water plate before the samples this is the most accurate way to blank.

5.3.5. AutoCalibrate: on. The instrument self zeroes against an internal reference.

5.3.6. Assay Plate Type: choose from the list of well types.

5.3.7. Strips: choose "read entire plate".

5.3.8. Speed Read: Choose no/off.

5.3.9. Column Priority: choose column priority

5.3.10. Autoread: off.

5.4. Click on Template to choose blanks, standards, and unknowns or just leave blank. The average of the Blanks will be subtracted from the data wells

5.5. Click on Reduction.

5.5.1. Click on absorbance and use both pre-read and path check.

5.5.2. Input any formulas you would like to use.

5.6. Click on Display to determine what data is shown (raw only or reduced).

5.7. Click on Temperature to set the assay temperature from 4^{o}C above ambient to 45^{o}C.

5.8. Place your blank plate in the reader.

5.8.1. Click the "drawer" button on the instrument to open and close the drawer.

5.8.2. Well A1 should be in the back left corner.

5.9. Click Read.

5.9.1. If a pre-read is being done, insert the blank plate and click Pre-Read.

5.9.1.1. The results for the pre-read will be "Path?"– this is normal.

5.9.2. Click Read again and choose "Normal" to read the real samples.

5.10. Your data will now appear in the plate display

5.10.1. Note that the linear range of absorbance is from 0-2.35.

5.11. You can copy and paste the results into excel for further analysis.

5.11.1. If you pre-read a plate, the results will appear in two sections:

The upper section is the raw result and the bottom section is the pre-read value.

Subtract the pre-read value from the raw result to get the corrected data.

5.12. To enter another plate click Experiment – New Plate .

5.13. To save the settings, click Save As.

this will save a .pda file (readable only in softmax).

5.14. Remove your plate, close the drawer, and log off from the computer when finished.