SOP: Varioskan LUX Plate Reader

Purpose: Quantify absorbance for serial dilution of food dye in microfluidic devices

NOTE: These are abbreviated instructions for the Varioskan LUX Plate Reader. The full manual for additional details and functionalities can be found on the website: https://assets.thermofisher.com/TFS-Assets/LCD/manuals/Varioskan-LUX-User-Manual.pdf

Location: BHE B8 (main area)

PPE: Flame-resistant lab coat; nitrile gloves; long pants; closed toe shoes

Protocol for Use:

1. Turn on the plate reader by flipping the switch on the left side. The instrument will take about 15-20 seconds to warm up and when ready, the plate slot will open and eject (**Figure 1**).
2. Double-click the “SkanIt RE 6.0.2” software on the desktop. The software will communicate with the instrument and run a series of quick calibration tests.
3. Click the “Home” tab and then the “New” icon. This will open a new session with notes, plate layout, protocol, results, and report.
4. Under “Notes”, include basic information about the experiment (e.g. group number, session number).

Figure 1: The plate reader with the plate slot open after initialization.

5. Then move to “Plate Layout” and start by adding the blank samples. Ensure that the “Blank” option is selected and then click and drag the cursor over the appropriate spaces.
6. Next, select the “Standard” option, and check the “Replicates” and “Concentrations” boxes.
    a. For “Replicates”, ensure that there are three columns and one row (3 x 1).
    b. For “Concentrations”, ensure that the “Series” option is selected, “First Value” is 1, “Operator” is division (/), and “Step by” is 2. Then click on the first well of where the 1:1 concentration will be placed on the 96-well plate and the triplicates will autofill adjacent to that space. The “First Value” will now be 0.5, which indicates that the next set of triplicates will be 1:2 concentration and so forth.
7. Continue to fill in all the standards until all serial dilution concentrations are accounted for.
    a. This example shows six concentrations (1:1, 1:2, 1:4, 1:8, 1:16, 1:32), but follow the directions in the protocol (**Figure 2**).

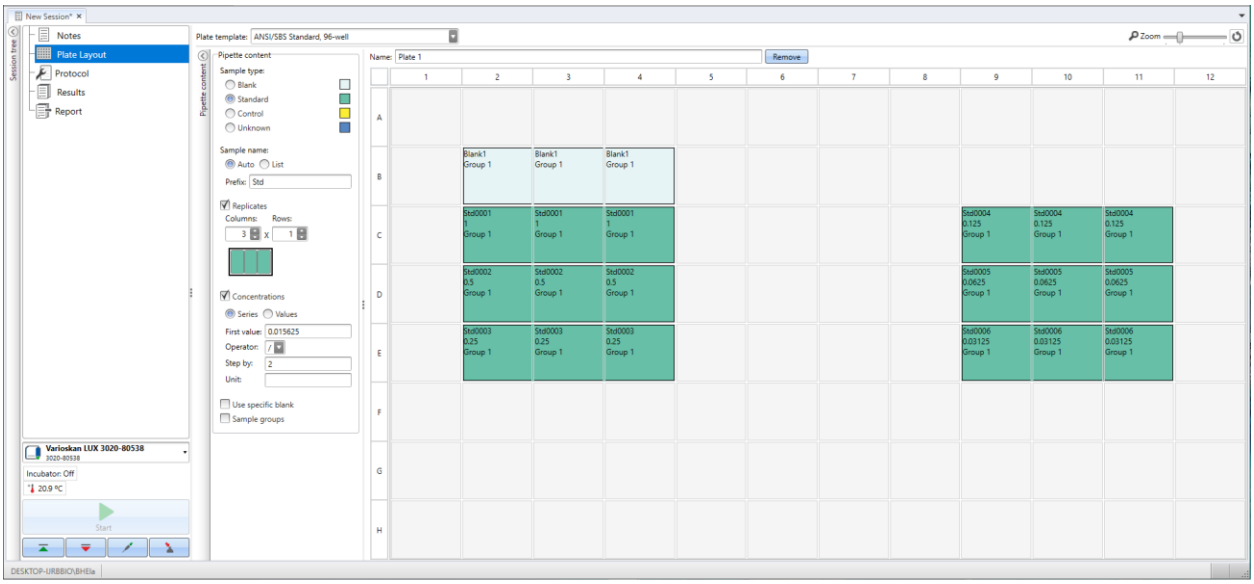

Figure 2: Example of the plate layout with six serial dilution concentrations.

8. Click “Protocol” and ensure that #3 is selected for “Measurement order”. Then select the “Absorbance” tab on the top of the page and set the “Wavelength [nm]” value to what is indicated on the protocol.
9. Click on “Absorbance 1” subsection below “Results” and select the “Blank Subtraction” tab on top of the page.
10. Click on “Blank Subtraction” subsection and select the “Standard Curve” tab on top of the page.
11. Double check to ensure that the plate layout, protocol, and results configurations are correct (**Figure 3**).
12. Ensure that the appropriate plate adapter is inserted in the plate slot (#2 for 96-well plate with no lid) prior to placing the plate on top.
13. Ensure that “Varioskan LUX 3020-80538” is selected and incubator is off. Click the “START” icon to begin the readings.
    a. Save the .skax file to the USB drive provided by the TA. Include group and session number in the file name.

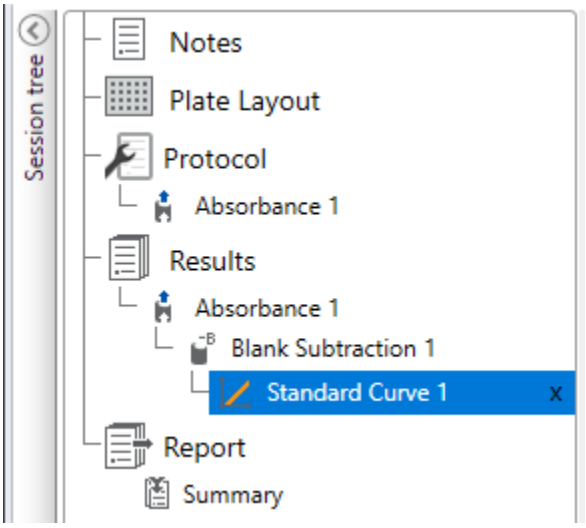

Figure 3: List of all components needed for the experimental session.

14. Click on “Absorbance 1” subsection below “Results” to see measurements appear. The process is complete when the plate slot opens and ejects.
15. Click on “Standard Curve” subsection under the “Results” section to see a standard curve (signal vs. concentration) (**Figure 4**).

a. The first row of numbers in bold correspond to the blank subtracted value divided by the average of the highest concentration values. The individual blank subtracted values are reported on the second row.

b. “> Max” denotes that the value is greater than 1.0.

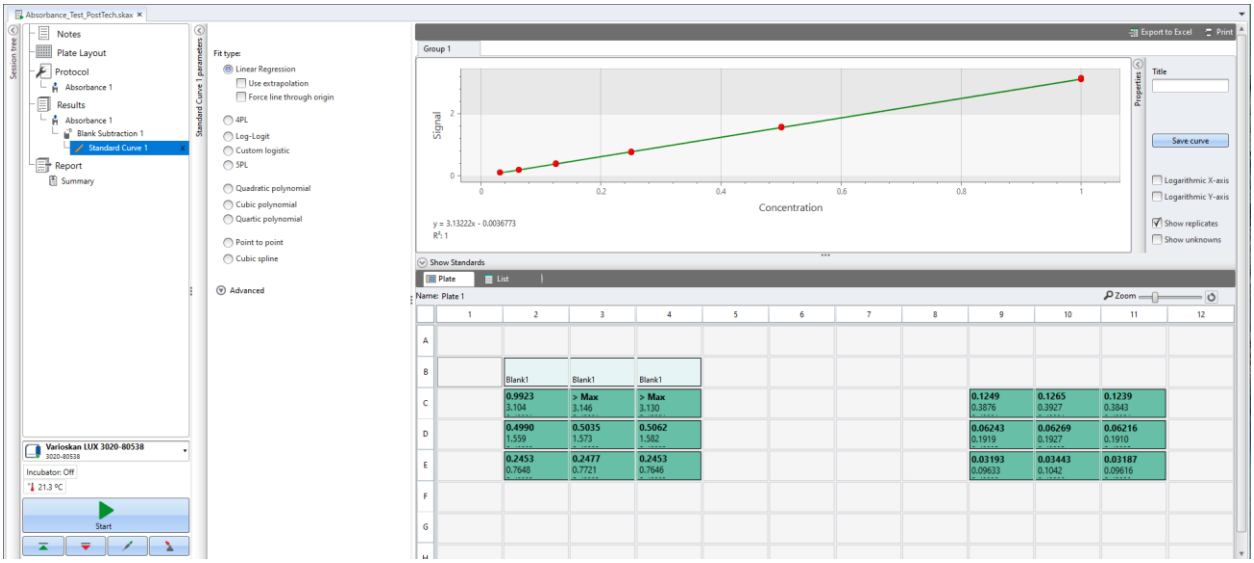

Figure 4: Standard curve generated for absorbance signal versus serial dilution concentration.

16. Save the plot and data by clicking “Export to Excel”. An Excel file should open with information regarding the absorbance measurements. Click “Save As” and save onto the USB drive.
17. Click the red eject icon to take the plate out and then click the green insert button to retract the holder and close the plate slot.
18. Close the SkanIt software after saving the data to a USB drive or folder on the computer.
19. Turn off the plate reader.

Maintenance Schedule:

Ensure that the plate reader is off after use.

Contact Information:

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