Plate Readers

PE Victor^3 (PSC 633, NSC 338)
Dispensor for Victor (PSC 633)

Every time you use the plate reader, you MUST sign the logbook.
Turn off the plate reader when you are done so the lamp doesn't burn out.

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PE Victor^3 V (Wallac 1420) Plate Reader

General Information

- The plate reader can be used for flash (Luciferase assays) or glow luminometry, fluorometry, fluorescence polarization, time-resolved fluorometry and photometry.
- This unit has a 2 syringe dispenser, shaking and temperature control.
- It is compatible with many plate types and if a plate is not available, it can be added to the plate list.
- The dispenser can add up to 2 substrates to wells. Substrate can be added to all wells (one at a time) and then read plate or substrate can be added a one well and read well before moving onto the next well.
- Make a new protocol when doing a new assay. Give protocol the name of the assay. Select appropriate filters. Now this protocol will be available in the drop down menu of the Workstation window. Make any changes (# and type of samples) for each new plate in the Start Wizard.
- Low signal – setup protocol to read longer.

Power on plate reader. Switch in the back on the right, just above the power plug.
Wallac 1420 Workstation (icon on desktop). Explorer (software to create/edit protocols) is accessed within the Workstation software.

Overview of menus and buttons. For more details of each item – see the manual.

Menus and Command

1. File. Only has exit.
2. Instrument. Start/Stop/End after plate (read is done).
3. Tools. Same items as buttons plus some additional choices.
   a. Explorer. To create/edit protocols
   b. Start Wizard. To edit an existing protocol for 1 run only. No permanent change to protocol.
   c. Results of latest assay run.
   d. **Labels (important to understand).** Defines labels used in measurements (excitation/Emission). Can add or copy a label; rename and edit label properties (lamp energy, excitation, emission, read time). The Labels window has 6 tabs: TFR (Time-Resolved Fluorescence), Fluorometry, Photometry, Luminometry, FP and LANCE. Depending upon which tab and label you select, there are different choices to edit (some have only 1 wavelength to choose from while others would have an excitation filter and an emission filter to choose. If windows are not enabled, you are looking at a factory pre-set label and these cannot be edited; just copy one, rename it and then edit.

i. New label. Can add or copy a label; rename and edit label properties. Select Excitation, Emission and read time. You also HAVE TO select CS-Lamp energy and control. The default is 0 which means if you don't adjust this, the laser will not be powered. CS-Lamp Control - use constant voltage for FP – fluorescence polarization; use Stabilized energy for Fluorescence. CW-Lamp Energy - start will 10,000, if signal is too low, turn up. Don't go too high, adjust read time if laser power is getting too high. You will need to optimize each new assay. Find the highest and lowest power values. Excitation and Emission aperture – this is how big a window over the well for a read; the larger the window, the bigger chance of crosstalk between wells.

e. Filters. Available Excitation filters and emission filters as well as their location on a wheel (excitation) or slide (emission). You can add, copy and remove filters as well as view their properties. 3 Tabs: Emission filters (list of available filters); CW-Lamp filters (list of available excitation filters); Filter Slides (select emission filter slide A to see the location of emission filters on the slide or select CW-lamp filter wheel A to see the location of excitation filters on the wheel. The other 2 choices for slide and wheel are designated B and are extra.) Add a new filter in the appropriate tab (Emission or CW-Lamp). New filter appears in the left panel of either Emission filter Slide A/B or CW-Lamp filter wheel A/B. Drag the filter to an empty slot on the slide or wheel.
f. EuSm Dual Label Normalization
g. LANCE Normalization
h. Plate Dimension Wizard. Add new plate to list.
i. Dispenser maintenance
j. Misc. Settings. 3 tabs: Plate type (add, copy, remove plate); Well names – add new well names, ex. Different std designations (add, copy and remove names); instrument (what instrument is available for software).
k. User Level.
l. Options. Shows what hardward and measurement technologies are installed.

Buttons From left to right under menu bar

1. Protocol Explorer. Create and edit protocols.
2. Start Wizard. Edit an existing protocol for 1 run only. This does not permanently change the protocol. Go through steps: select protocol, change well names (blank, measured, std, etc.), add notes. Clicking Finish will start the plate read.
3. Latest assay run. View results of last run.
4. Dispenser Maintenance
5. Help

Tabs

1. Instrument Control. Drop down menu of existing protocols. Select one. Start; Stop; End (after plate read) buttons. Status window shows what step in the protocol.
2. Live Display. Results in plate format. Move cursor over well to see number. Color codes at right show size of results. Measurement information shows information about sample being measured. This display only shows results of the first measurement. If there are multiple measurements, you will need to go to Latest assay run button to see all the results.
3. Temperature. Inside chamber temperature ($15^{o}C$ – $45^{o}C$). Be sure to select Apply if temperature is changed. Temperature vs. time is shown in a graph. Temperature cannot be controlled through the protocol.

Run

1. Open lid and load plate.
2. Place A1 in the top left corner of the chamber.
3. Select protocol
4. Select Start from the button or Edit drop down list.
5. You can run multiple plates

6. Use the Start Wizard to indicate how many plates and where your samples are for this particular run. Use this Wizard so that you don't have to create or edit a protocol for each run. Selecting Finish at the end of the Wizard will start the read.

Explorer. Create and Edit Protocols.

1. Open Wallac 1420 workstation. Open Explorer - 1st button.
2. Open Wallac folder to see all available factory set protocols. All of these have a lock icon, so they cannot be edited. Copy (left click) whichever protocol you would like to use.
3. Open your lab folder. Left click and paste. Rename protocol to reflect your assay.
4. Double click on new protocol to access Protocol Editor.

Protocol Editor

1. Samples. Highlight appropriate wells for your expt. Right click to select type of sample: blank (well becomes white), measured (well becomes dark blue), standard (well becomes pink), or empty (well becomes gray). Go to Tools/Misc. settings in the Workstation to add different well names). You can also setup more than 1 plate with buttons that allow you to add, copy or delete a plate. Select the upper left hand corner of plate (triangle) to select all the wells. Drag the mouse to select entire rows and columns. Shift and drag mouse to select a rectangle of wells.
2. ID. Name your protocol and/or pick a number for your protocol. Add any notes you would like for the assay.
3. Measurement. Measurement sequence in protocol. CPS = counts per second. Different operations are available depending on what protocol you are editing. This is where you select a label, shake, setup a dispensing step, set a delay/incubation time, kinetics (readings over time) and scan (different wavelength readings on same samples).
   a. Mode. Specifies what unit (single well, strip or plate) the complete measurement sequence should be performed before moving onto the next similar unit.
   b. Operations.
      i. Right click in empty window – new, paste. New allows different labels and other operations.
      ii. Right click in selected operations – copy, paste and properties of operation.
      iii. Up and down arrows. Enabled when more than 1 operation added to list. Can move an operation one step up or down.
      iv. Delete. Deletes a selected operation.
      v. Once the following are set, you can view operation properties in the right hand window.
      vi. Dispense. When using the dispenser, use this button to open a window to select which dispenser syringe, volume of dispense, speed, increment (100 ul minus to 100 ul plus; volume added or subtracted from the previous volume dispensed before the next dispensing), # replicates (1 -3) and injection mode (can either aspirate and dispense the same amount [aspvol=dispvol] or you can fill the whole syringe and then dispense several times [aspvol=syringevol]; the former is more accurate but the latter is faster). Perform on 1st plate repeat only – check if you want dispensing for the 1st of the plate repeats; uncheck to dispense for every repeat.
      vii. Delay. Duration of delay (0.1 – 3600 sec.) between the preceding operation and the start of the next operation. (i.e. incubation time). Perform on 1st plate repeat only – check if you want dispensing for the 1st of the plate repeats; uncheck to dispense for every repeat.
      viii. Shake. Parameters for a shake – time, speed, the extent of the move and type of shake (linear, orbital or double orbital). Perform on 1st plate repeat only – check if you want dispensing for the 1st of the plate repeats; uncheck to dispense for every repeat.
      ix. Label. If plate is selected in the measurement mode window, you can add up to

10 labels (link to definition above). Double click on Measurement line to see properties.

x. Kinetics. One well at a time for entire set of reads Kinetic measurement – up to 100 measurements and the delay between each measurement (0 – 600 sec.); also select label button. You can only use this button using well mode.

xi. True Kinetics. See #c. Plate for setup for a true kinetics expt.

xii. Scan. Several measurements for the same well. Select a label (button). You can only use this button using well mode. This compares to the Spectrum selection on the SpectraMax plate reader.

xiii. Dispenser head control. Controls injector head. This is used for very fast Kinetics and can only be used in well mode.

c. Plate. True Kinetics. Plate Repeat Parameters - Specify # of times for each plate to be measured and amount of time between each measurement. Plate type – you can change type of plate. If yours isn't on the pull down list, you can add one in Wallac 1420 Manager/Tools/plate dimension Wizard. Temperature level checking – will make sure that plate is at specified temperature before reading.

d. Output. Decide what information to print. Select what folder to put the Excel Results page and also what information is included in the Results.

e. Events.

f. General. Information about protocol – dates created, etc.

Results. To view results.

1. Latest Assay Run button
2. Explorer. Select folder and assay. Each run will come up in the right hand panel. Double click to view the results in Excel.
3. Note the tabs at the bottom. You can view results in list mode and plate mode.

Using Victor Dispenser

1. Cleaning and Filling dispenser tubing before using Dispensor
   a. Open Dispenser window (icon or Tools/Dispenser Maintenance)
   b. Place 2 lines of tubing in 70% EtOH
   c. Flush both lines (select buttons 1 and 2) 70% EtOH
   d. Flush both lines (select buttons 1 and 2) H2O
   e. Take tubing out of H2O and hold tubing over waste cup
   f. Empty both lines
   g. Fill both lines with reagents. Put tubing in appropriate reagents. Fill 500 ul with both reagents. Prepare 1 ml – there is a large void volume
2. Recover reagents
   a. Place appropriate dispenser tubing back into each reagent tube.
   b. Open Dispenser window (icon or Tools/Dispenser Maintenance)
   c. Empty both lines into the tubes.
   d. Place 2 lines of tubing in 70% EtOH
   e. Flush both lines with 70% EtOH
   f. Flush both lines with H2O
   g. Take tubing out of H2O and hold tubing over waste cup
   h. Empty both lines

Optional: Flush with 70% EtOH for about 30 minutes. You can do this OR Core facility will do this ~ once a week if the instrument is used.