BMG CLARIOstar Plus Microplate Reader

Standard Operation Protocol

I. Basic Specification

Detection Modes	- Fluorescence Intensity – including FRET - Luminescence – including BRET - UV/Vis Absorbance - Time-Resolved Fluorescence
Measurement Modes	- Top or Bottom optic - Endpoint or Kinetic (fast or slow) measurements - Spectral Scanning (Fluorescence, Luminescence and Absorbance) - Well Scanning
Readable Range	- Fluorescence Excitation: 320-740nm - Fluorescence Emission: 350-850nm - Luminescence: 320-740 nm - Absorbance: 220-1000nm
Atmospheric Control	- O_2 range: 0.1-20% - CO_2 range: 0.1-20% - Temperature range: 25ºC-45ºC
Reagent Injector	- Up to two built-in reagent injectors - Injection into 6 to 384 well plates - Individual injection volume for each well (3 to 500µL) - Up to four injection events per well

II. Plate Format

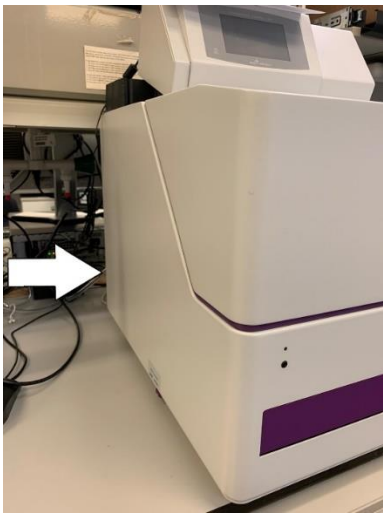
The reader can read any 6- to 1536-well plate with SBS standard, or non-SBS standard-that fits max. diminsion:128x86x20; min: length 124. Optimal microplate color shall depend on the measurement mode. Microplate material shall not have similar wavelength absorbance peak as the sample.

Fluorescent Measurement	
Top optic	Black microplates with solid bottom
Bottom optic	Black microplates with transparent film bottom or glass bottom
Luminescence Measurement	
Top optic	Solid white microplates
Bottom optic	White microplates with transparent film bottom or glass bottom
Absorbance	
-	Transparent microplates

III. Initialization

1. Turn on the microplate reader by the On/Off switch at the back.

   **Make sure do NOT switch off the instrument when the status light is flashing!!



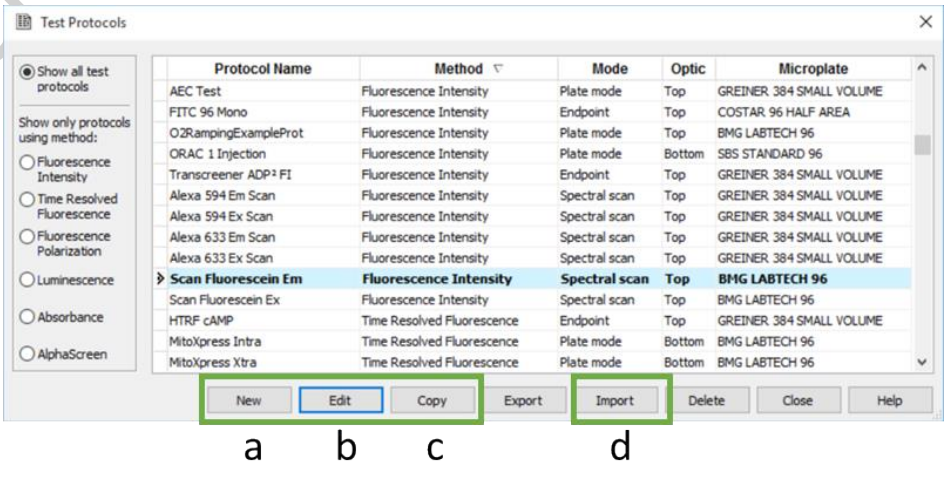
2. Log in PPMS tracker.
3. Open the control software.
4. Login to the control software with your username and password created in training.

IV. Fluorescence/ Luminescence/ Absorbance Measurement

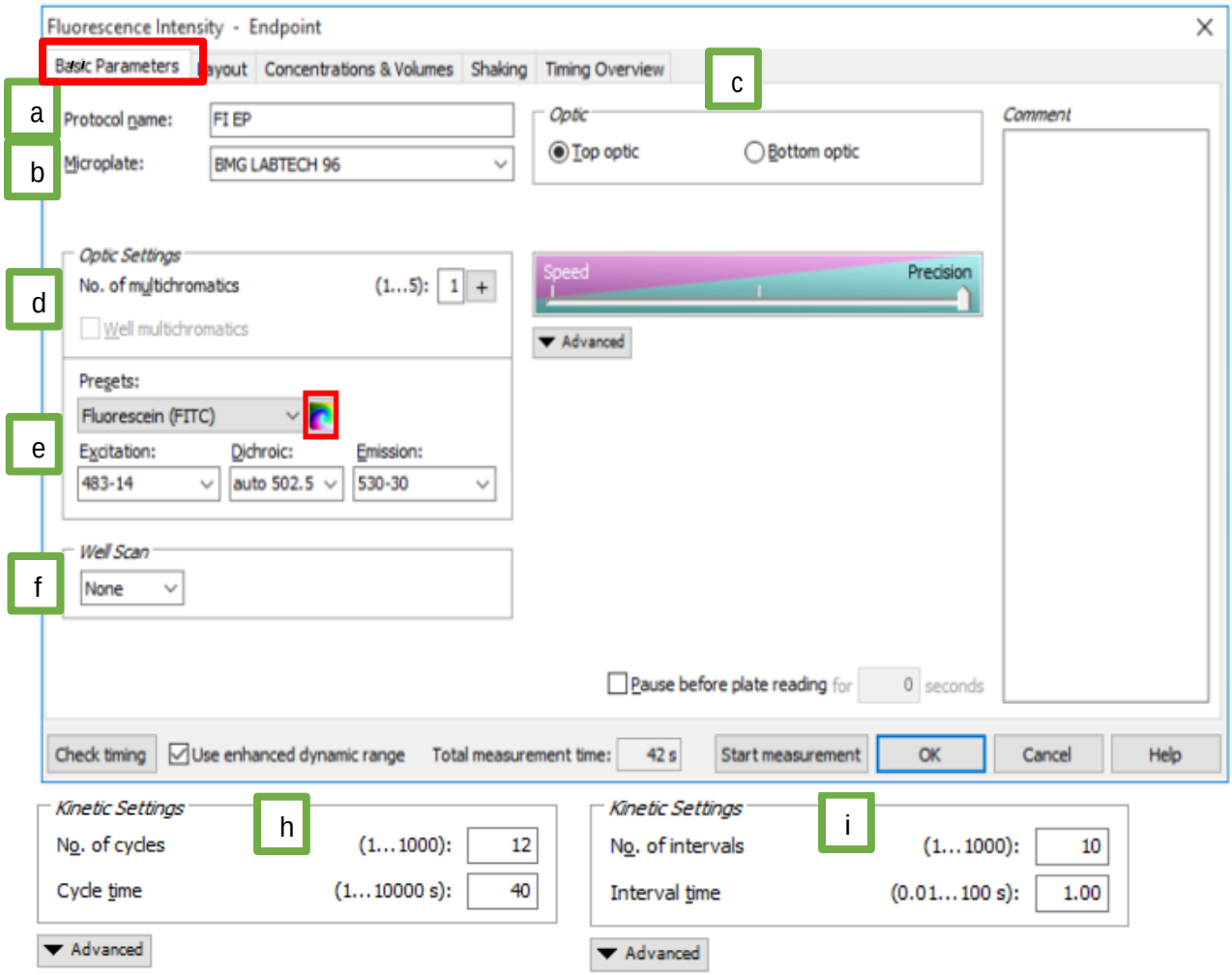
1. To edit existing test protocol or define new ones, click on 'Manage Protocols'.

You could open a test protocol by clicking:

a. ***New*** – create a new protocol
b. ***Edit*** – select a protocol from the list and click 'Edit' to edit on existing protocol
c. ***Copy*** – select a protocol from the list and click 'Copy' to copy an individual protocol from the existing one
d. ***Import*** – import protocol created using CLARIOstar software (*.TSC) from outside source



2. Select suitable reading method: *Fluorescence Intensity; Luminescence; Absorbance*.
3. Select suitable reading mode: *Endpoint/ Plate mode/ Well mode/ Spectral scan*.
   - **Endpoint:** Measure the plate for once
   - **Plate mode:** Measure the plate for more than one time (usually for slow kinetics)
   - **Well mode:** Measure each well individually for defined number of times consecutively (usually for fast kinetics)
   - **Spectral scan:** Measure spectra instead of discrete wavelengths
4. Enter '***Basic Parameters***'.



a. **Protocol name:** Name protocol (suggested name pattern: "Stain(/targeted molecule)"_ "Ex/Em".
b. **Microplate:** Select corresponding microplate format. Please inform CPOS staff if your microplate is not found in the list.
c. **Optic:** Select optic (top/bottom). Top optic should suit most of the cases; bottom optic could help if molecules emitting light resident at the bottom of the wells.
d. **Optic Setting:** Enter no. of dye to be detected.

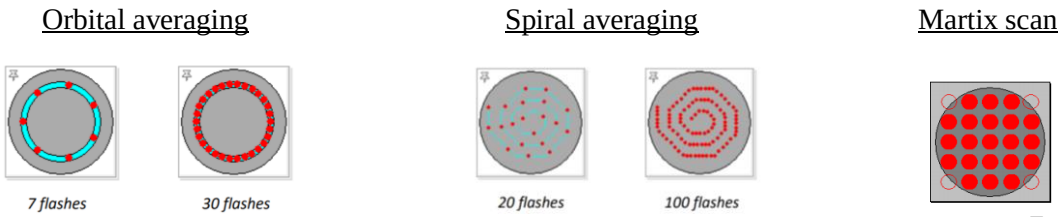
   ☑ *Well multichromatics*: measure all chromatics in one well before moving to another

   ☐ *Well multichromatics*: measure the first chromatics for all wells before the second chromatics

e. **Presets:** Select the fluorophore(s) to be detected (preset default excitation and emission combination will be shown). Excitation and emission wavelength can be modified by typing numbers directly. Modified preset will be shown in purple with a star (*).

   Or choose combination from installed filter list/type in the desired wavelength-width.

f. **Well Scan:** Collect and average signals from different points instead of the middle to improve result for non-homogenous samples.

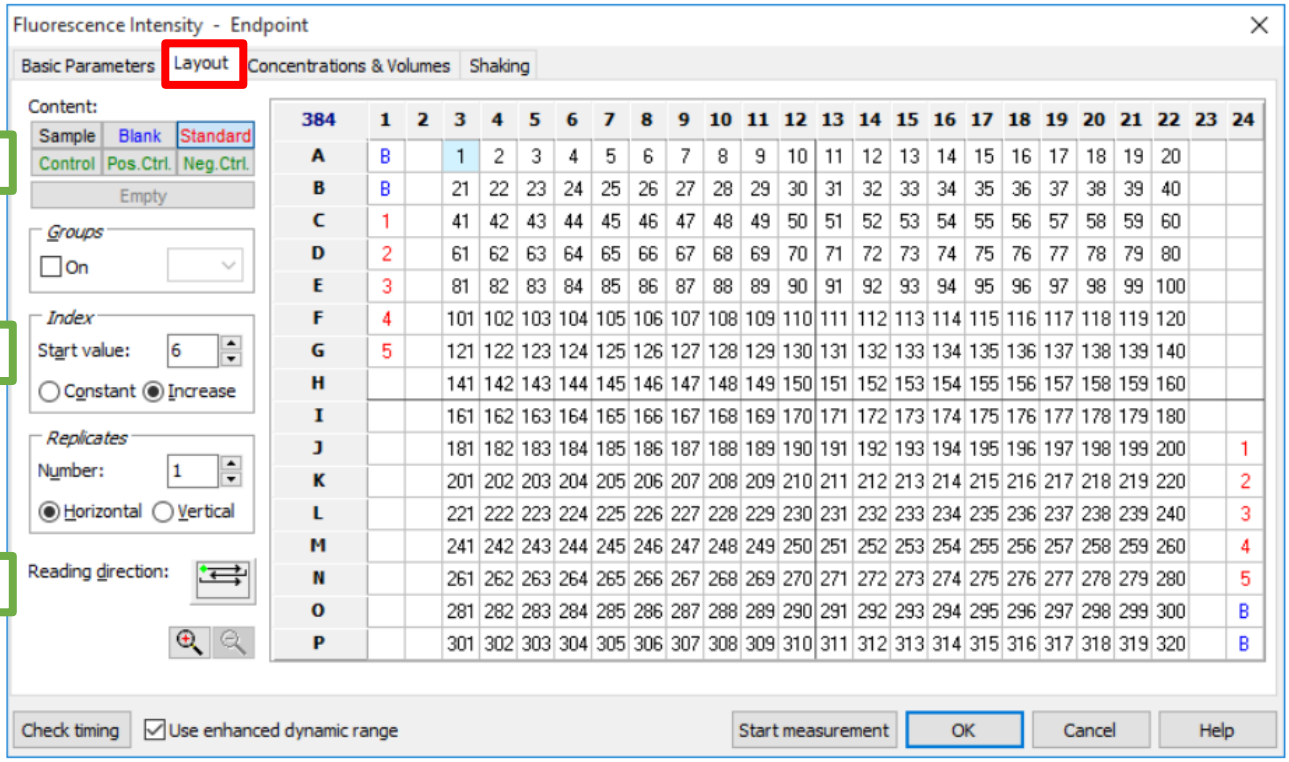


g. **Speed vs Precision:** Adjust *settling time* and *no. of flashes* if necessary.

h. **Kinetic settings [Plate Mode]**: Enter *No. of cycles* (number of times the entire plate will be measured) and *Cycle time* (the time it takes to measure the plate during one cycle)

   Kinetic settings [Well Mode]: Enter *No. of intervals* (number of times the well is measured) and *Interval time* (the time in seconds that each measurement interval should last)

5. Edit '***Layout***'.

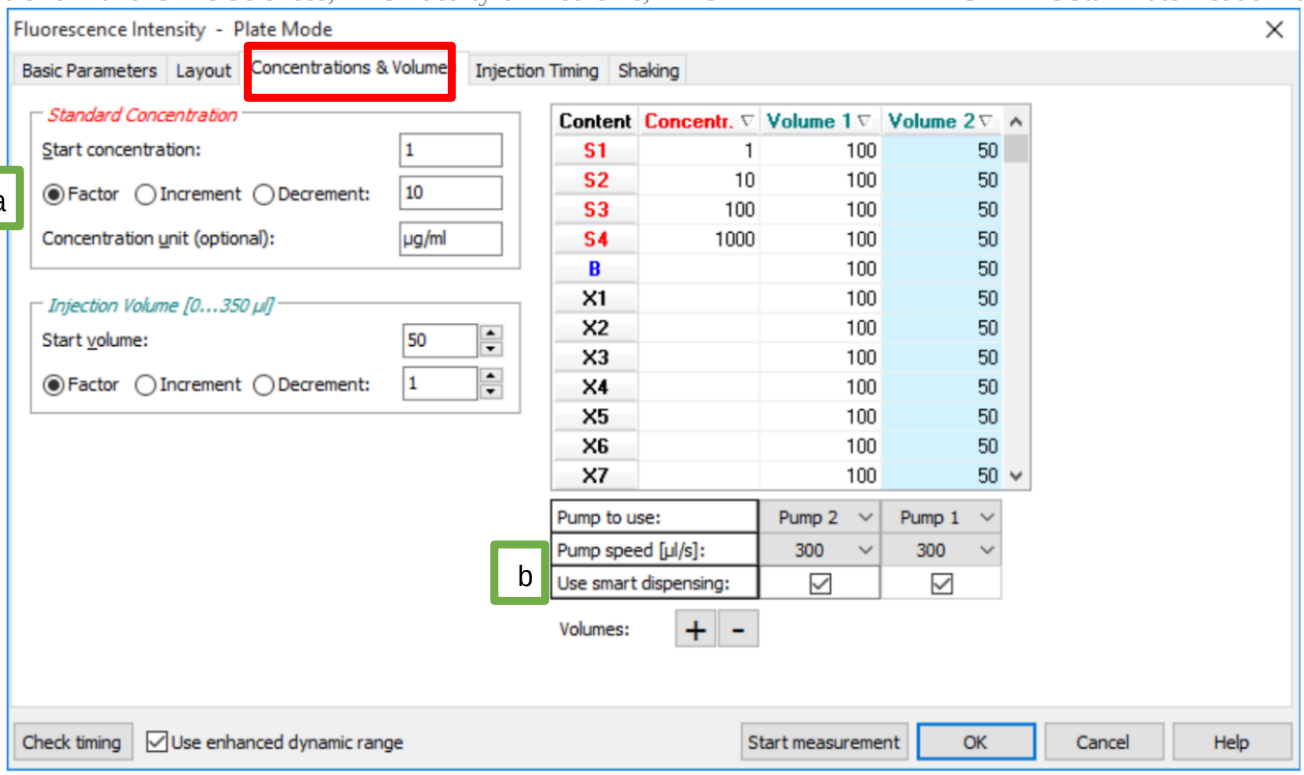


a. **Content:** Select appropriate content type. Then, either double click on a well or click and drag across wells to label with the content type.

b. **Index:** Select *Constant* for replicate labeling or select *Increase* for consecutive number labeling.

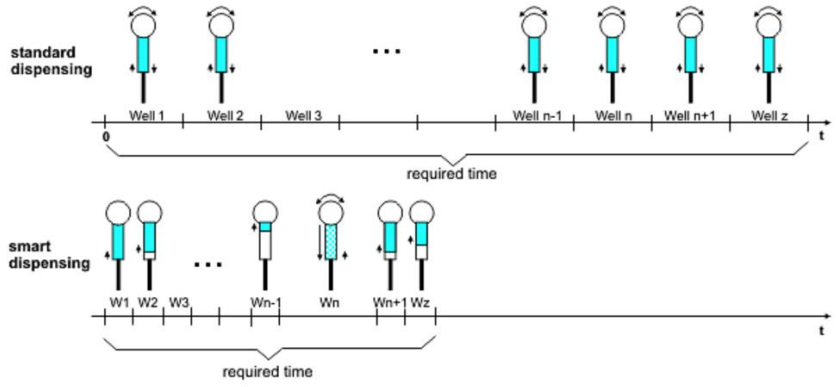
c. **Reading direction:** Choose reading sequence (horizontally/ vertically/ bidirectionally/ unidirectionally).

6. [Optional] Edit '***Concentrations & Volumes***'.



a. Fill in *Standard Concentration* and *Injection Volume* boxes, then click and drag across cells wanted to be filled. Values can also be entered manually.

b. ☑ *Use smart dispensing* if you wish aspiration of injection liquid only occurs when remaining volume in syringe is smaller than the injection volume, instead of aspirating before every injection to reduce time needed.

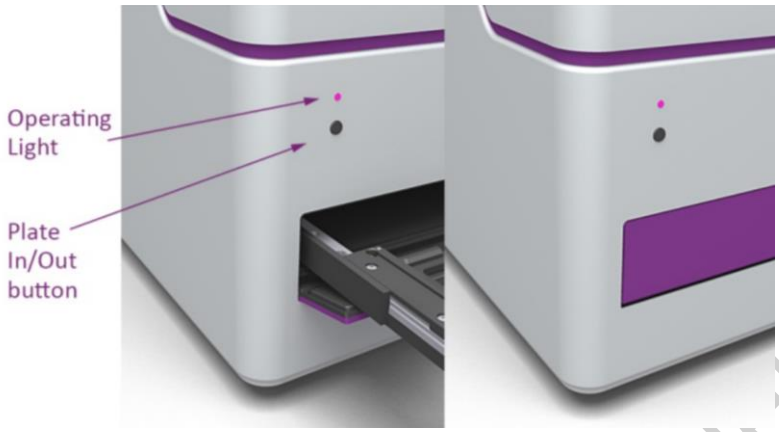


c. ☑ *Equidistant injection* [Endpoint mode]: Ensure same time difference between injection and measurement for each well.

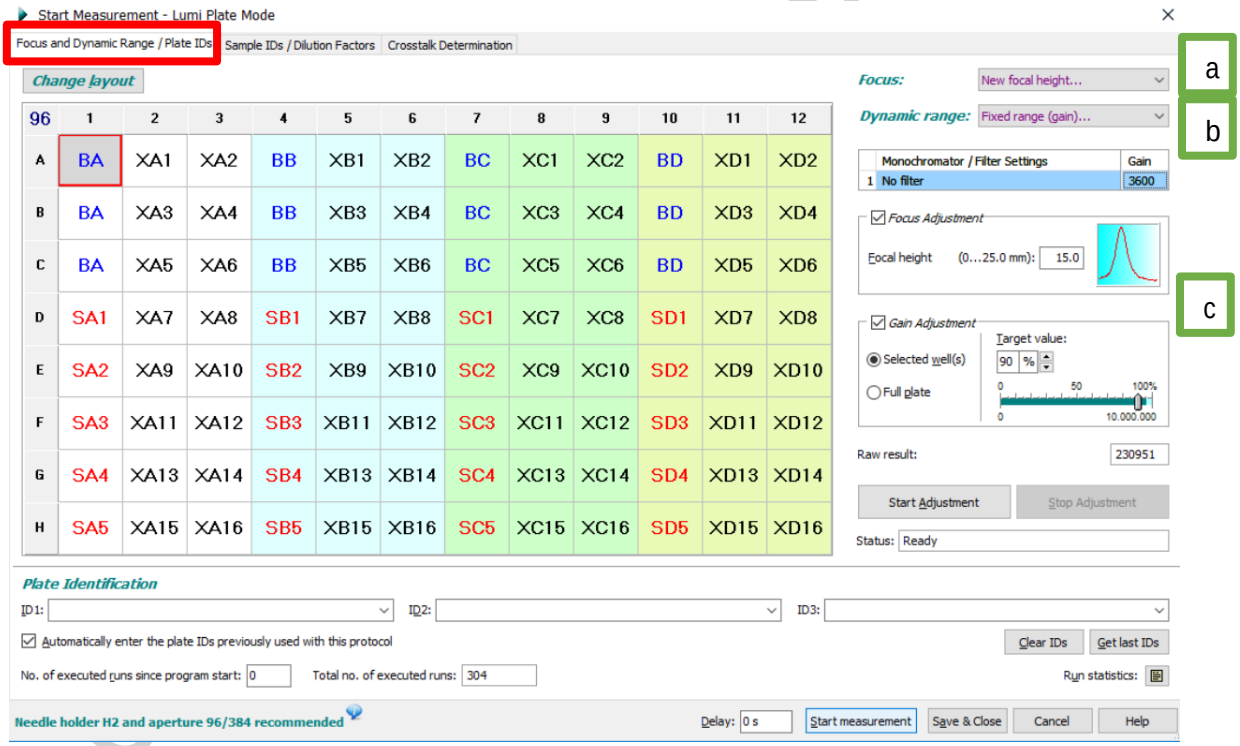
7. [Optional] Input '***Injection Timing***'.
8. [Optional] Define shaking actions in 'Shaking' (Recommended:300rpm). The frequency of shaking should be lower for larger wells or cell-based sample.
9. Click *Check timing* box in lower left corner to calculate the total measurement time.

** If you are performing injection, follow steps in **Injection preparation** before going to the next step.

10. Press the plate In/Out button to move the plate carrier out. Cover your plate with a lid if your sample contains organic solvent or bacteria. Put the plate on the carrier with well position A1 on the upper left corner.



11. Press the plate In/Out button to move the plate carrier in.
12. Click *Start measurement.* 
13. Edit '***Focus and Dynamic Range/Plate IDs***'.



a. Select *Focus* in the drop-down lists.

   Autofocus: Auto detect optical focal height of the well with highest signal

   Use Previous focal height: Use previously defined height for reference

   New focal height: Define new height by *Focus Adjustment*

b. Select *Dynamic range* in the drop-down lists.

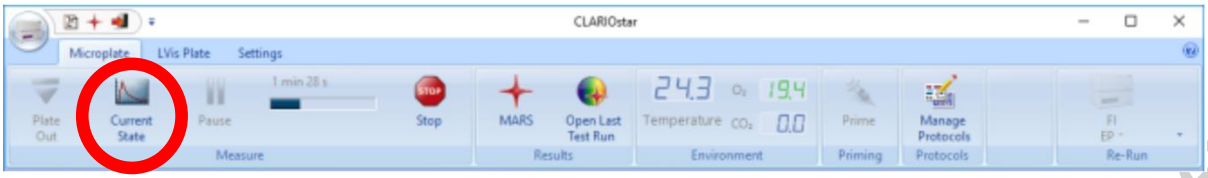
   Fixed range: Use fixed gain value for the whole plate

   Enhanced dynamic range (EDR): Suitable gain will be adapted automatically for different wells

c. To define new *Focal height* and *Gain*, ☑ *Focus Adjustment* and ☑ *Gain Adjustment*, then click *Start Adjustment* to decide the optimal values

   Or type in the numbers manually

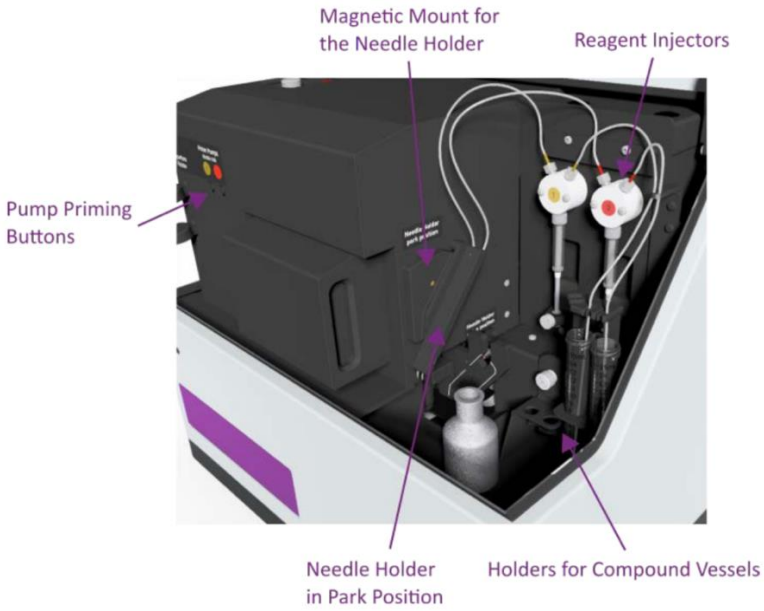
14. Click *Start measurement* when every setting is ready.
15. During the measurement process, you could click on *Current state* to view raw measurement data concurrently.



16. A notification sound is emitted when the measurement is finished.
17. Follow *Quick Guide CLARIOstar MARS Data Analysis Software* for simple analysis.

V. Injection preparation (Handle needle with care!!)

****Please inform CPOS staff for the substance planning to inject beforehand****



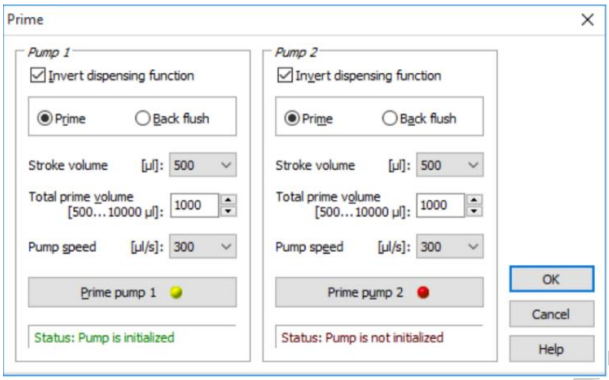
1. Open the plate reader lid. Put the tube of fluid (15ml tube) into the *Holder for compound vessels*. Remember to contain adequate fluid (including priming and injection).
2. Clean the plastic tubing with ethanol-wetted kimwipe and immerse into the fluid. Be aware of which injector(s) you are using.
3. Place a waste container **OUTSIDE** of the instrument and hold the needle holder by hand over the waste container so that priming waste can go into the container.
4. Open prime window in the software.

5. Enter prime volume and speed. The recommended total prime volume using water/ethanol is 1000uL while the recommended minimum total prime volume using reagent is 500uL to wash the whole syringe.

   ☑ *Invert dispensing function*: begin injection with filling up the syringe then dispensing

   ☐ *Invert dispensing function*: begin with dispensing remaining volume in syringe then refilling

   Then click 'Prime pump 1/2' to start priming.



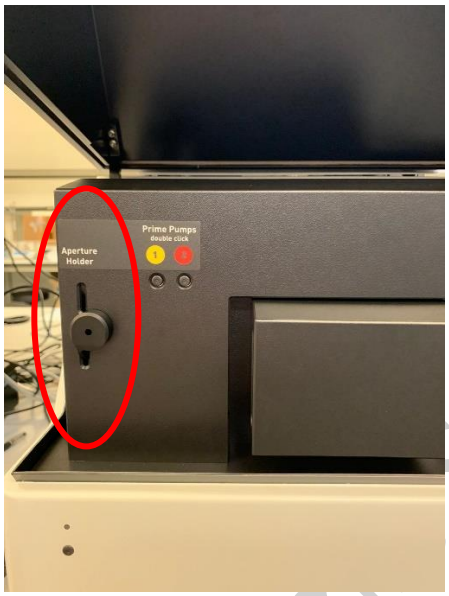
6. After priming, attach the needle holder to active position, close the plate reader lid and you could execute injection and measurement.

Injector Cleansing (after use)

1. Take the tubing out from the fluid container and cleanse with ethanol-wetted kimwipe.
2. Put a tube of excess distilled water into the vessel holder and immerse the tubing in.
3. Apply prime with distilled water to flush the syringe thoroughly (recommended: stroke volume 500, total prime volume 2000) outside the reader into the waste container. Repeat this priming step with 70% ethanol and then 100% ethanol.
4. Withdraw the tubing from the tube of water/ethanol and apply prime to flush out any remaining solutions.
5. Remove the distilled water/ethanol tube and return the needle holder to parking position.

VI. Aperture Spoon

1. An aperture spoon could be used in luminescence measurement with 96/384 well plates to reduce crosstalk between wells.
2. When not using, the spoon is attached as the below photo inside the lid.

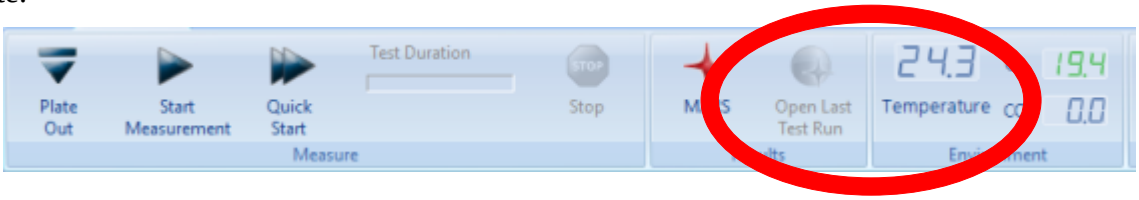


3. To use the spoon, snap it on to the right side of the needle holder, as shown in below picture.

4. Attach the needle holder to working position and close the lid.
5. Return the spoon to *Aperture holder* after use.

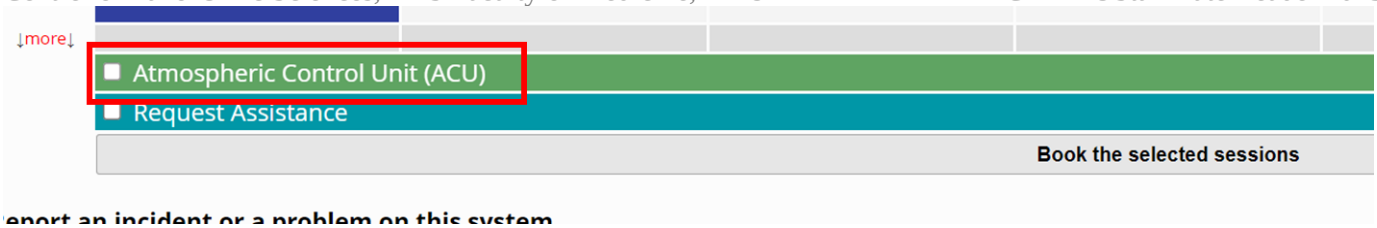
VII. Temperature incubation

1. Set desired temperature of incubation (25-45°C) in Temperature menu command. Click *Incubator On* to activate.

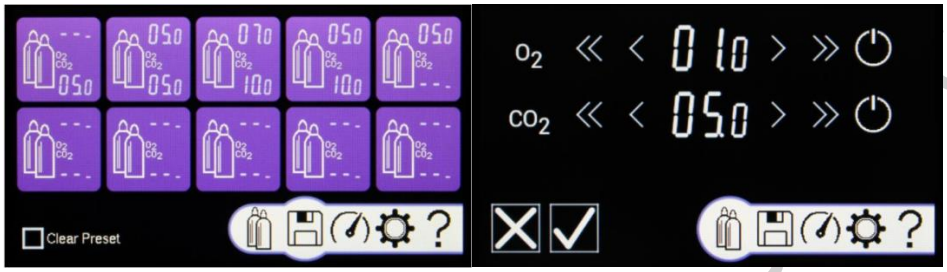


Atmospheric Control Unit (ACU)

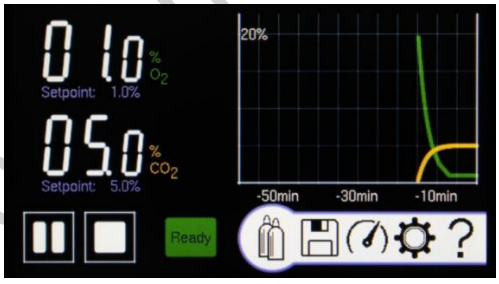
** ACU is of extra charge at $15/hr. Please check the option of "***Atmospheric Control Unit (ACU)***" when you book the reader on our system and email the detail to us.



1. Turn on the ACU and the desired gas cylinder(s) to be used.
2. Tap on 
3. Select a preset gas mixture or select an empty one and adjust desired CO_2 concentration. Tap  to confirm.



4. If you need to pause or stop gas flow. Tap  on and tap either 
5. The adjustment can also be made in the CLARIOstar software as mentioned in step 2.
6. Depending on the concentration, settings stabilization typically takes 15 to 30 minutes. Plan and book extra time for stabilization before run. 'Ready' in green will be shown on the status screen when the stabilized status is reached.



VIII. Turn Off

1. Take out any plate or tube loaded.
2. [Optional] Turn off the ACU by the power button on the bottom left corner of the control panel and turn off the used gas cylinder(s).
3. Wait till the operating light (beside plate in/out button) remains constantly purple.
4. Turn the microplate reader off by the On/Off switch at the back.
5. Transfer data
6. Log out PPMS tracker.
7. Sign log book.