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General Operating Procedure for: Spark microplate reader

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Contents

1. Purpose

The purpose of this GOP is to regulate the operation, usage and maintenance of spark microplate reader.

2. Scope

This GOP applies to all personnel using and managing spark microplate reader.

3. Procedure

3.1 Switching on the instrument

3.1.1 Press the start button (fig.1) in the instrument back.

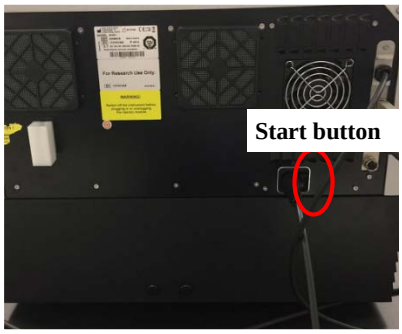


Fig.1 Rear view of the instrument

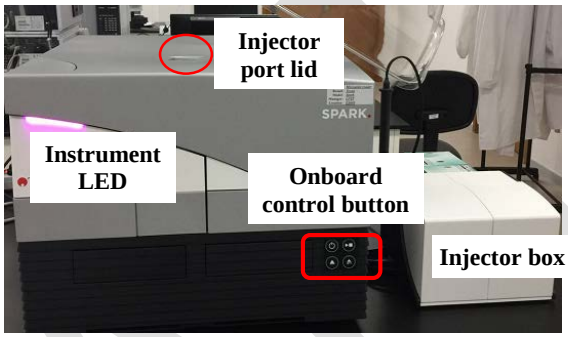


Fig.2 Front view of the instrument

3.1.2 Power on the computer, the pass word to log in is “xjtlu123”.

3.1.3 Wait till the instrument LED emit purple light (fig.2). Double-click the icon of Sparkcontrol – dashboard 

. After connection, the Dashboard displayed on the software interface (fig.3).

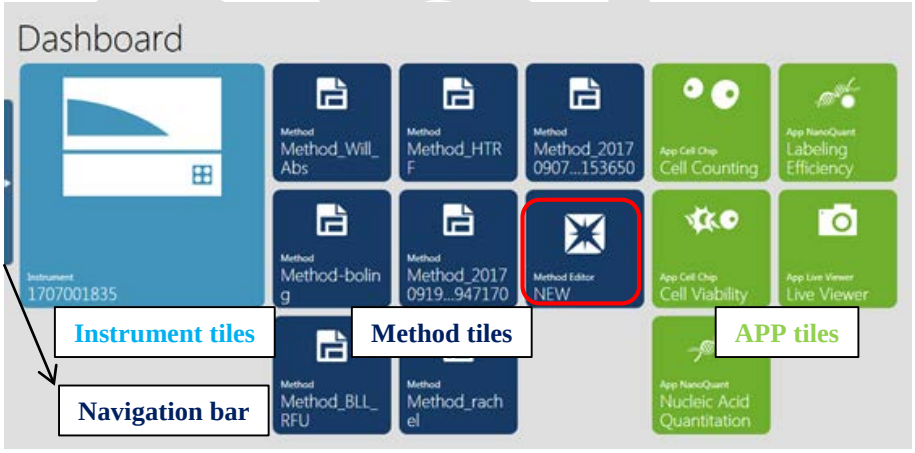


Fig.3 Dashboard

3.2 Setting up a method

Skip this part if using existed method.

Click  (fig.3) on dashboard to enter method editor.

3.2.1 Defining the plate

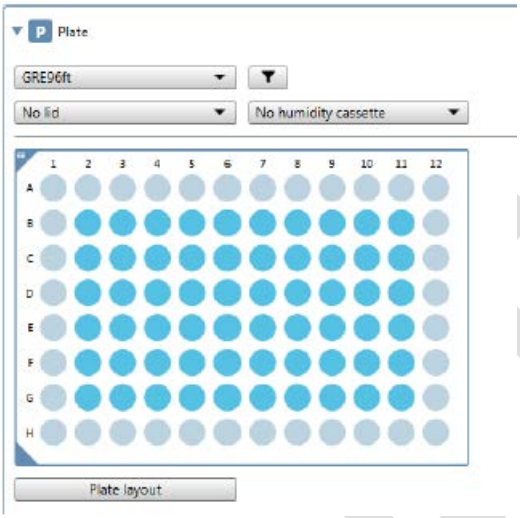


Fig.4 Plate

Plates are specialized for different applications; please make sure you are choosing the right plate by checking the plate manual.

Select the plate to be used for the measurement. Click  to filter the list of available plates. Also select the options concerning plate lid and humidity cassette.

Caution: a removable lid is used in combination with the lid lifter. Please make sure to attach a magnetic pad to the plate lid before use.

3.2.2 Defining the plate layout (not necessarily)

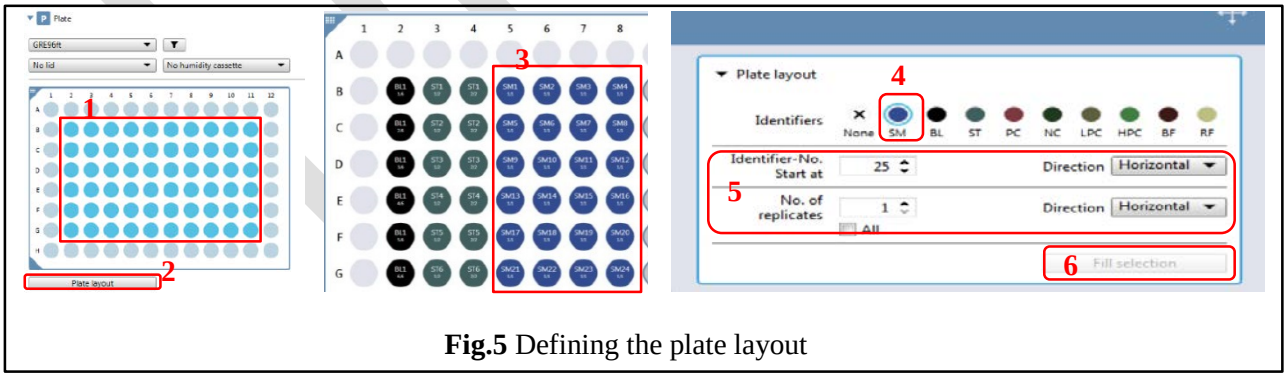


Fig.5 Defining the plate layout

Check below for the meaning of different identifiers: BL (blank), SM (sample), ST (standard), PC (positive control), NC (negative control), LPC (low positive control), HPC (high positive control), CL (calibrator), RF (reference), BF (blank for polarization reference).

3.2.3 Defining the strips

Double click a strip to insert the strip to work flow pane and define each strip after read corresponding chapters carefully. Please refer to the manual of reagent kit or reference papers for parameters setting. Please also check the advanced settings for each strip.

There are 4 kinds of strips:

- **Plate strips:** plate, part of plate, well
  Use Part of Plate to define sub-areas within the previously selected plate area. Well strip is used to perform measurement well by well.
- **Action strips:** shaking, wait, injector, condition, temperature, move plate, user intervention and comment.
  For detailed information about action strips, refer to *3.4 action strips* on page 7.
- **Detection strips:** absorbance, absorbance scan, florescence intensity, florescence intensity scan, TR fluorescence intensity, luminescence, luminescence multi-color, luminescence scan, cell counting and cell confluence.
  We will introduce absorbance (3.5, page 9) and florescence (3.6, page 10) later in this manual. Please make sure to check corresponding parts of the manual before define the strip. For other applications, please talk to Yili Cheng for further information.
- **Kinetic strips:** kinetic loop

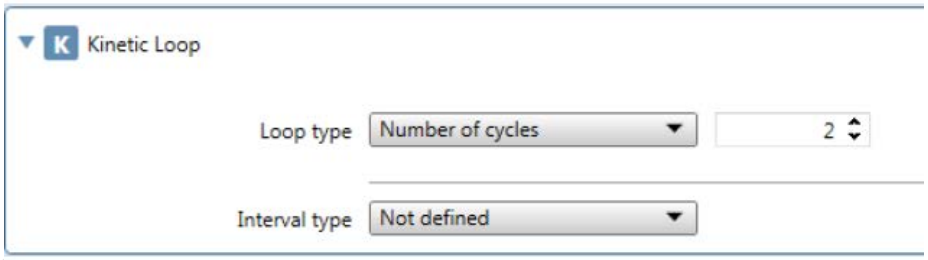


Fig.6 Kinetic loop strip

Some explanation:

Loop type	Select number of cycles and define a cycle; select duration and define duration of the kinetic measurement.
Interval type	Select Not defined for measurements without a kinetic interval time, measurements are performed as fast as possible. Select Fixed for measurements with a kinetic interval time and define the interval time.

Note: To enable the options Continuous shaking and Continuous waiting define a kinetic measurement with a fixed interval time.

3.2.4 Hierarchy of strips

Any desired measurement step can be inserted directly after a Plate, Part of Plate or Well strip. You can modify the sequence of execution of a single strip by right clicking the strip and selecting outdent or indent in the pop-up menus (fig.7).

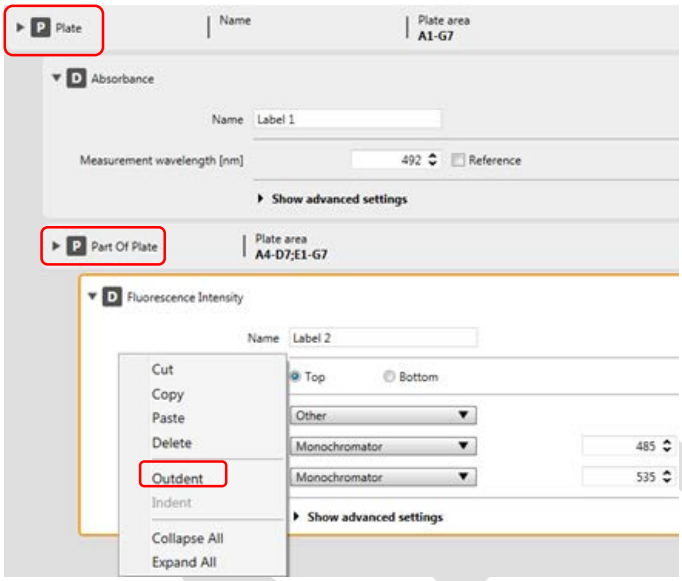


Fig.7 Hierarchy of strips

The actions of all strips with the same indentation are performed sequentially. A strip that is indented more than the previous strip shows dependence between the two strips. This means that the parameters defined in the first strip are also active for the second (indented) strip.

3.2.5 Save method

Click the save button on the toolbar (fig.8) on top or the method editor.

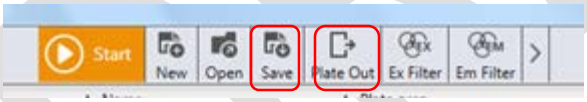


Fig.8 Toolbar

3.2.6 Plate control

Click 

on the toolbar or press the onboard control button  .

Insert the microplate, the position of well A1 has to be on the upper left side (fig.9). When loading samples, the filling volume can't exceed the maximum volume claimed on the plate definition file.

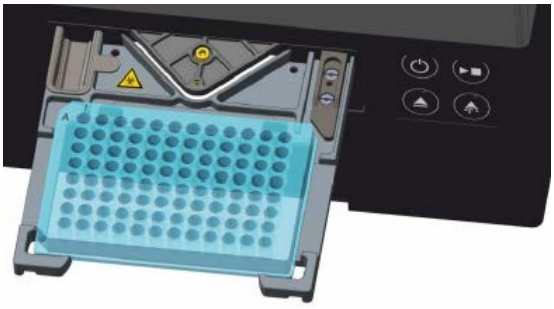


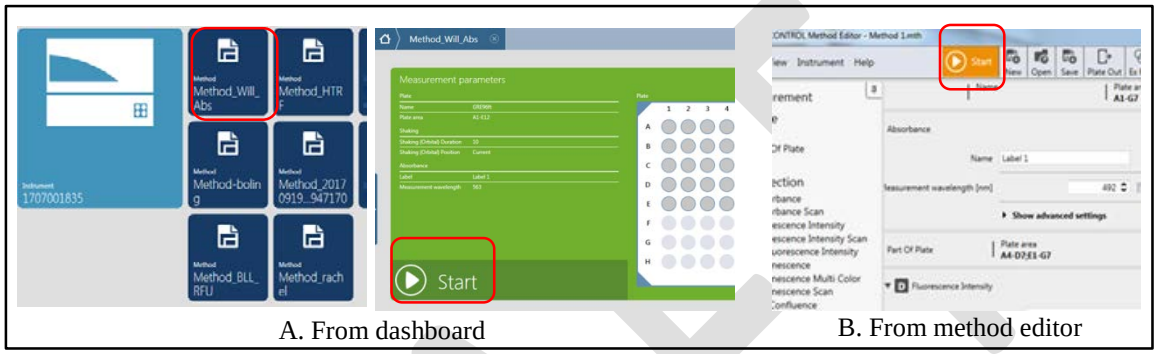
Fig.9 Microplate on the plate carrier

Caution: make sure no potential fluorescent or luminescent contamination lies on top of the plate as droplets. Wipe up any spill immediately with absorbent material and dispose of contaminated material appropriately.

Press  again to make the plate carrier in.

3.3 Starting a method

A method can be started directly from the method editor or dashboard (fig.10).



A. From dashboard B. From method editor

Fig. 10 Starting a method

3.4 Action strips

Insert corresponding strips according to your demand.

3.4.1 Shaking strip

Set each parameter according to your experiment condition or reference papers.

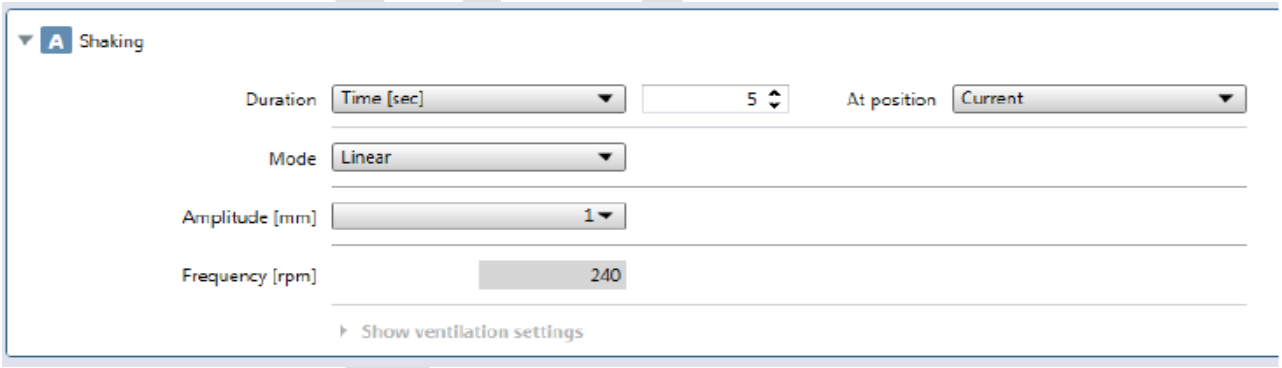


Fig.11 Shaking strip

Some explanation:

At position	Current with ventilation or incubation with ventilation could only be selected when removable lid or humidity cassette is selected. If you also need temperature control, the plate must be placed in incubation position while shaking to keep the temperature constant and provide uniformity across the plate.
Amplitude	1-6 mm (humidity cassette not selected); 2.5-6 mm (humidity cassette selected).

Show ventilation settings	Select the interval time for ventilation in minutes; select the duration of ventilation in seconds.

3.4.2 Wait strip

This strip can be used to define a specific time period before the next step within a workflow is executed.

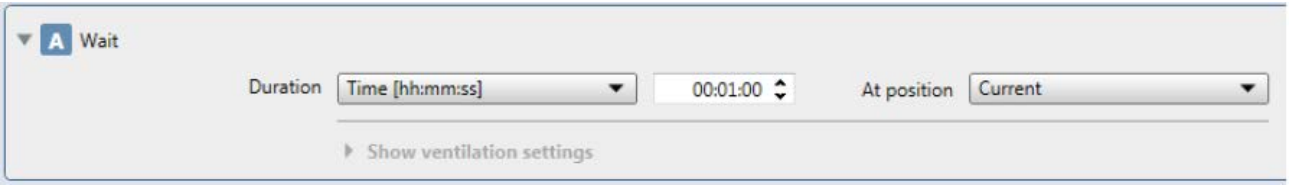


Fig.12 wait strip

Some explanation:

Duration	Continuous waiting could only be selected and used within a kinetic measurement with a fixed interval time.
At position	Select incubation position if need to control the temperature.

3.4.3 Condition strip

Command: start at cycle to perform the conditional step at a defined cycle; start/stop at value to execute/stop the conditional step at a defined raw data value.

3.4.4 Injector strip

Please carefully read chapter *3.7 using injectors* on page 12 before using this strip.

The injectors can be used alone or in combination with the following detection modes: Fluorescence Intensity top and bottom, Time Resolved Fluorescence, Fluorescence Polarization, Absorbance, Luminescence as well as Multicolor Luminescence.

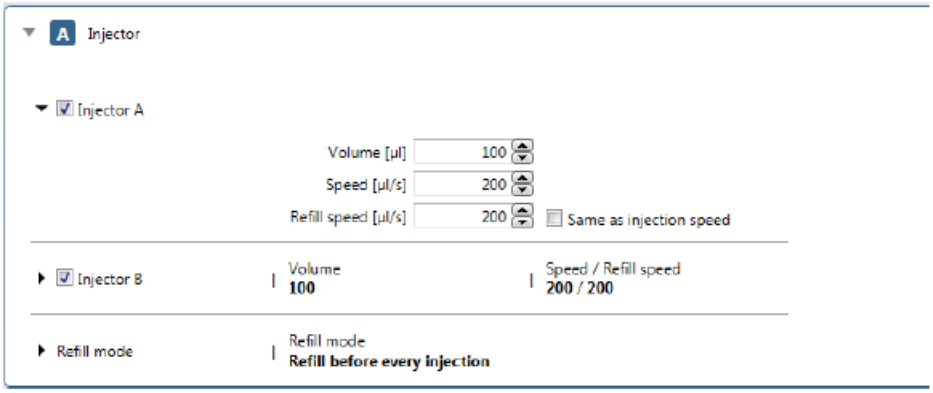


Fig.13 Injector strip

Explanation:

Refill	Standard: As soon as the liquid in the syringe is used up, the syringe is refilled with the

mode	defined refill volume.
	Refill before every injection.

3.4.5 Temperature strip

The heating module enables temperature control within a range from 3 °C above ambient temperature to 42 °C. If not incubated externally, the microplate should be left for equilibration before the measurement is started.

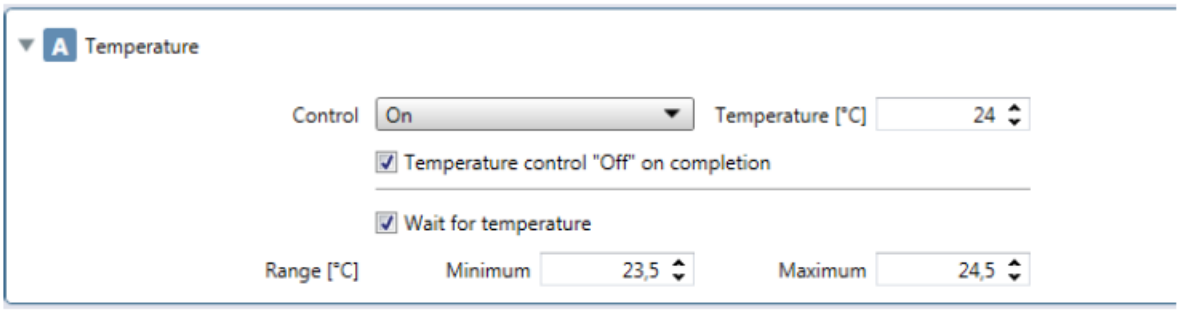


Fig.14 temperature strip

The temperature control can also be switched on manually via the Temperature Control window in the Method Editor.

Select Temperature control. Enter the Target Temperature and click “Set” to start heating (fig.15). Clear the Temperature control check box to stop heating.

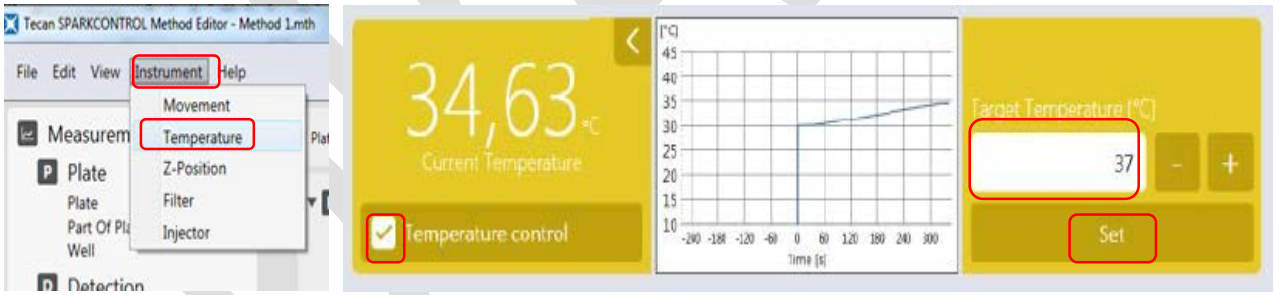


Fig.15 Temperature control window

Note: When starting a method with temperature control, the method settings will always overrule the manual settings if their definitions do not match.

3.4.6 User Intervention Strip

The User Intervention strip informs the operator of the instrument to execute a particular action during the workflow at a specific time. A message appears and the measurement process stops until OK is clicked.

3.5 Absorbance

Absorbance applications can be performed at any wavelength from 200 to 1000 nm.

Caution:

- Use UV compatible microplates (transparent or UV- transparent) for absorbance measurements in UV wavelength range.
- To obtain more accurate measurement data avoid OD-values above 3.

OD (Optical Density): 1 OD means 10-fold light attenuation, i.e. 10 % transmission. 2 OD means 100-fold light attenuation, i.e. 1 % transmission

3.5.1 Absorbance strip

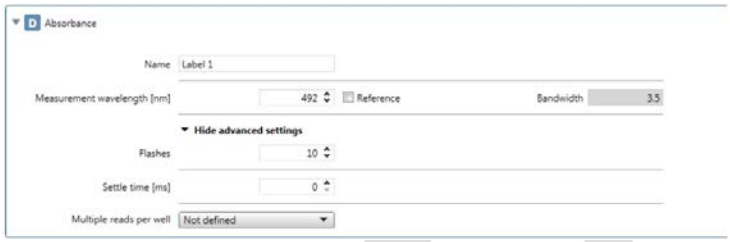


Fig.16 Absorbance strip

Explanation:

Band width	3.5 nm
Flashes	For optimal performance, use the default number of flashes.
Settle time	Due to the stop and go motion of the plate carrier the liquids meniscus may vibrate during signal integration. It is recommended to select a settle time between move and flash between 100 and 300 ms.

3.5.2 Absorbance scan

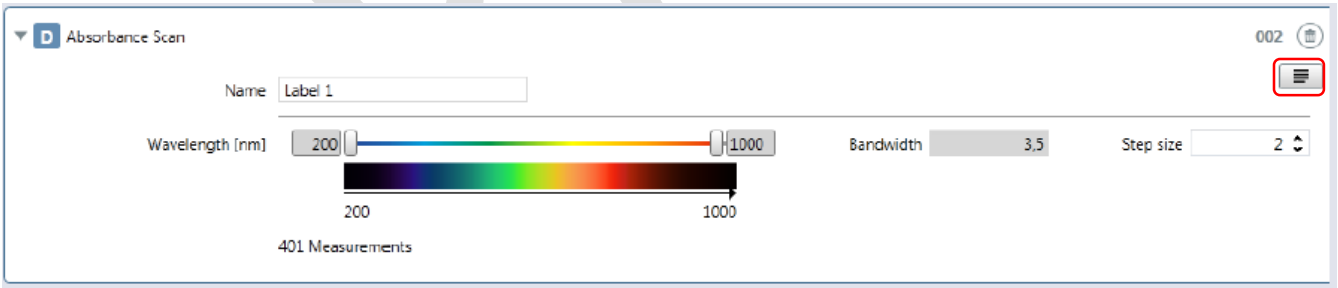


Fig.17 Absorbance scan

Define the wavelength range by set the from and to value or by moving the respective slider.

3.6 Fluorescence

3.6.1 Fluorescence intensity strip

Explanation:

Mode	Top	Used for reagent sample or suspension cell.
	Bottom	Recommended for adherent cells. Make sure the bottom of the microplate is transparent.

flushes		The recommended range is 25 to 35.
Gain	Manual gain	Define a gain value to be used for the measurement (range: 1-255).
	Optimal gain	Recommended for all applications that produce results with unknown RFU values.
	Calculated from well	The optimal gain value is calculated for the selected well. The resulting gain value is applied to all other wells within the selected well range.
	Extended dynamic range	The optimal gain measurement is done in two consecutive parts. Select this option to optimally adjust the gain settings for very high and very low signals on a microplate within one single measurement.
	Use gain regulation	Available for plate-wise kinetic measurements only. Permit the measurement of even very high signals.
Z- position	Manual	
	Calculated from well	
	Same as	Select a label in order to set the Z-position of the current label equal to that of the selected label.

3.6.2 Time- resolved fluorescence intensity strip

The settings of different parameters are almost the same as fluorescence intensity strip, except that it require a lag time and increased integration time according to the particular application.

Lag time: time between flash and the start of signal integration.

Integration time: duration of signal recording per well.

3.6.3 Fluorescence intensity scan strip

3 scan modes: excitation scan, emission scan and 3D scan. The start value of emission wavelength should be larger than the end value of excitation wavelength.

For other parameters refer to fluorescence intensity strip.

3.6.4 Fluorescence polarization module

Explanation:

Smooth mode	Suggested to open for 2 to 24-wells microplate.
G-factor	Depends on wavelength and fluorophore. Can be set manually. Need to be recalculated if wavelengths/ filters have changed or different fluorophore is used.
Reference	Use the well containing fluorophore and buffer solution. (RF or SM)
Reference blank	Containing the buffer solution without fluorophore (BF or BL).
Mirror	Select Dichroic 510 or automatic.
Z-position	Recommended to select calculated from well.
Settle time	300

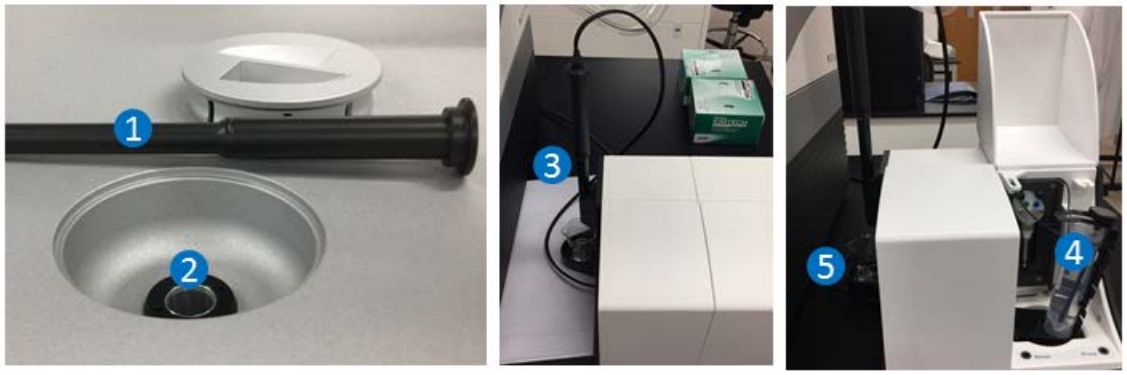
3.7 Using injector

Skip this chapter if do not need to use injector.

Caution:

- Make sure that the injector carrier is in the service position for rinsing and priming (fig.18).
- Please refer to appendix 1 for reagent compatibility.

3.7.1 Before injection



1. Injector dummy
2. Injector port
3. Injector carrier in service position
4. Feeding tubes in storage bottle
5. Waste container

Fig.18 Rinsing and priming

3.7.1.1 Make sure that the injector carrier is in the service position and remove the injector dummy out of injector port.

3.7.1.2 Rinse:

a. Fill the storage bottles with the appropriate reagents (deionized water, 70 % ethanol …) and insert feeding tubes of the injector system. Make sure that the tube(s) reaches the bottom of the bottle.

b. Put an empty container under the injector.

c. Press the Rinse button on the injector box to start the rinsing procedure or from the software. (Define all the rinse parameters and click start backflush to start fig.19).

d. Click close to exit the injector/ rinse window if using the software to control.

Fig. 19 Rinse parameters

3.7.1.3 Prime:

It is recommended to perform a rinsing step before priming.

The steps are almost the same with rinse, expect that:

1. The regent maybe different;

2. Press the prime button on the injector box or define the prime parameters on software instead of those of rinse;

3. Visually inspect the liquid jet, the syringes for air bubbles and the tube(s) for leaks and kinks. Any bubbles should be removed after priming to ensure good injection performance.

3.7.1.4 Press the injector carrier gently into the injector port to lock it in place.

3.7.1.5 Fill the storage bottle with appropriate regent and insert the feeding tubes to the bottle before the injection.

3.7.2 After measurement

3.7.2.1 Remove the injector from the carrier port and insert it into the service position of the injector box.

Warning: Hold the injector carrier only by the handle provided for this purpose.

3.7.2.2 Reagent backflush:

The steps are almost the same with rinse; expect that reagent backflush could only be conducted by software through the injector/ backflush window.

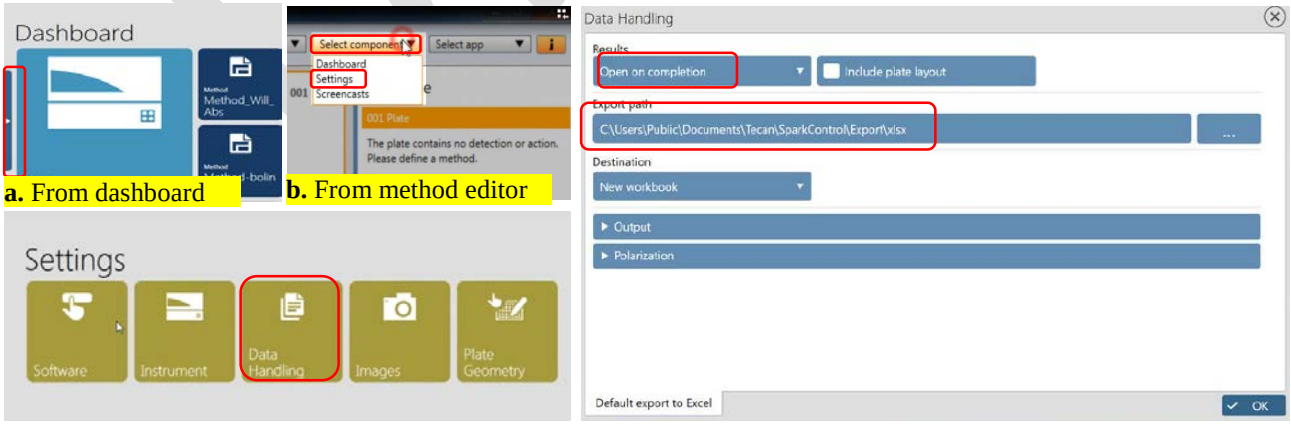
3.7.2.3 Rinse:

Perform a rinse procedure to clean the injector system.

3.7.2.4 Keep the injector at service mode and insert the injector dummy into the injector port.

3.8 Data export

The results are saved automatically after the measurement was completed and the output settings could be changed by clicking Settings> data handing. Settings could be targeted either from dashboard or method editor.



a. From dashboard **b.** From method editor

Fig.20 Data handling

Note: If the defined export path can't be accessed by the software, the results will be exported to C:\Users\Public\Documents\Tecan\SparkControl\Export\xlsx\AUTOSAVE.

3.9 Shutting down the instrument

3.9.1 Press the onboard control button to exit the microplate.

3.9.2 Turn off the instrument and computer.

4. Appendix

Appendix 1 Reagent compatibility of injector

Please refer to the following list for reagent compatibility. Rating 'A' indicates a good compatibility with the injector system. Chemicals with the rating 'D' must not be used with the injector system. They will severely damage the injector system.

'A' Rated Chemicals	D' Rated Chemicals
Acetic Acid < 60%	Acetonitrile
Dimethyl Formamide	Butyl Amine
Ethanol	Chloroform
Methanol (Methyl Alcohol)	Carbon Tetrachloride (dry)
Water, Deionized	Diethyl Ether
Water, Distilled	Ethanolamine
Water, Fresh	Ethylene Diamine
Potassium Hydroxide (Caustic Potash)	Furfural
Potassium Hypochlorite (aqueous)	Hexane
Sodium Hydroxide (< 60%, aqueous)	Hydrofluoric Acid
Sodium Hypochlorite	Monoethanolamine
	Sulfuric Acid (diluted or concentrated)
	Tetrahydrofuran

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