

How to use Flex Station3

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□=Basic operation , Red Number=Important operation , Black number=Advanced Operation

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How to use absorbance mode

wavelength range:200nm~1000nm , (Max) 6 point simultaneous measurement

Corresponding plate:6~384well plate

1. Turn on the plate reader power.

(Warning : All of the door opens and closes. Sometimes, you will hear a loud sound.)

2. Turn on the PC , and login windows.

3. Wait until the temperature is displayed. (about 3min?)

(Does not recognize the device.)

4. Double-click on the desktop of “SoftMaxPro” icon.

(Warning : The door opens. In order to close the door, press the “reading chamber” button.)

5. Click the “Setting” button in the “Plate01” tab.

【6】. Set the following items, click OK.

Read Mode : Absorbance(ODs)

Wavelengths : Lm1=Measurement wavelength (Numerical entry)

Assay Plate Type : Usually , Select “xx Well Standard clrbtm (ABS)”

xx=The number of wells

7. Press “reading chamber” button. Remove the lid , and put the plate.

(If the lid is necessary, please use the seal. When you use a deep plate, please remove the spacer.)

In order to close the door, press the “reading chamber” button.

8. Start the measurement by clicking the “Read” button.

When the measurement is finished plate is ejected.

【9】. Value is displayed on the "Plate1" tab. Drag the well image , and press Ctrl+C.

10. To start the excel , and press Ctrl+V. Save the worksheet.

11. When you continue the measurement, go to step 6.

12. When the measurement is completed, exit the “SoftMaxPro” software.

Exit the Windows, and turn off the plate reader power. Please fill in the used note.

<2 wavelength measurement method (subtraction)>

Add to step 6.

Wavelengths:1→2 , Lm1=Measurement wavelength , Lm2=Reference wavelength

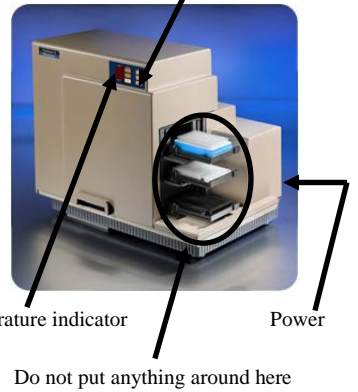
Add to step 9.

Click the “Reduction” button. Select the “Lm1-Lm2” in the “Wavelength Combination” field. Click OK.

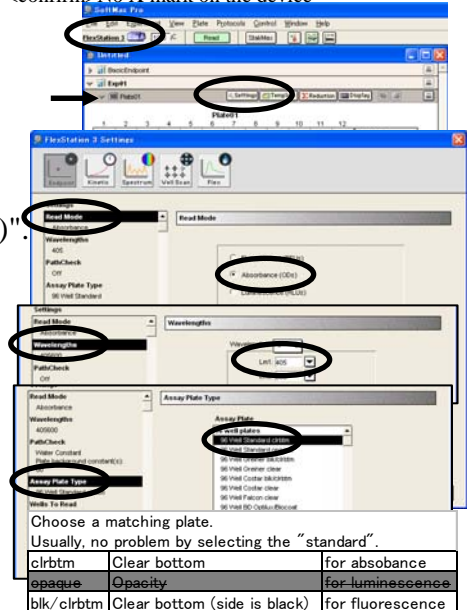
Click the “Display” button. Select “Reduced” , and click OK. (The difference is displayed.)

Warning : Value copied to Excel is the display value.

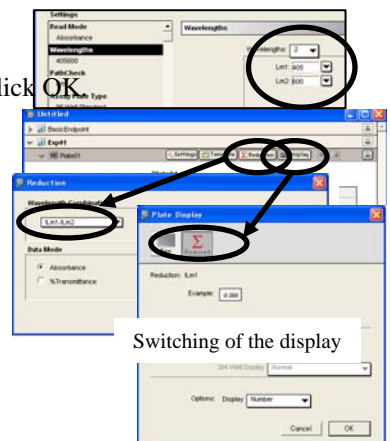
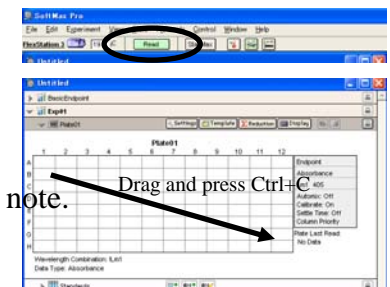
reading chamber
(Opening and closing of the chamber)



<confirm>No X mark on the device



clrbtm	Clear bottom	for absorbance
opaque	Opacity	for luminescence
blk/clrbtm	Clear bottom (side is black)	for fluorescence

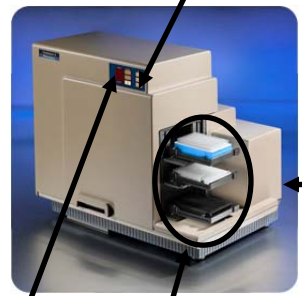


How to use fluorescence mode

wavelength range:250nm~850nm , (Max) 4 point simultaneous measurement

Corresponding plate:6~384well plate

reading chamber
(Opening and closing of the chamber)



Temperature indicator

Power

Do not put anything around here

1. Turn on the plate reader power.

(Warning : All of the door opens and closes. Sometimes, you will hear a loud sound.)

2. Turn on the PC , and login windows.

3. Wait until the temperature is displayed. (about 3min?)

(Does not recognize the device.)

4. Double-click on the desktop of “SoftMaxPro” icon.

(Warning : The door opens. In order to close the door, press the “reading chamber” button.)

5. Click the “Setting” button in the “Plate01” tab.

【6】. Set the following items, click OK.

Read Mode : Fluorescence(RFUs)

(When you check the "Read from bottom", measured from the bottom.)

Wavelengths : Input excitation and emission wavelength.

Assay Plate Type : Choose the ones that you use.

Clear plate→clrbtm , Black plate→opaque

7. Press “reading chamber” button. Remove the lid , and put the plate.

(If the lid is necessary, please use the seal. When you use a deep plate, please remove the spacer.)

In order to close the door, press the “reading chamber” button.

8. Start the measurement by clicking the “Read” button.

When the measurement is finished plate is ejected.

【9】. Value is displayed on the "Plate1" tab. Drag the well image , and press Ctrl+C.

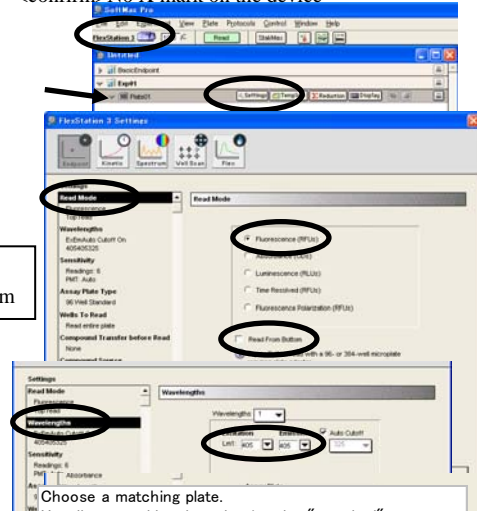
10. To start the excel , and press Ctrl+V. Save the worksheet.

11. When you continue the measurement, go to step 6.

12. When the measurement is completed, exit the “SoftMaxPro” software.

Exit the Windows, and turn off the plate reader power. Please fill in the used note.

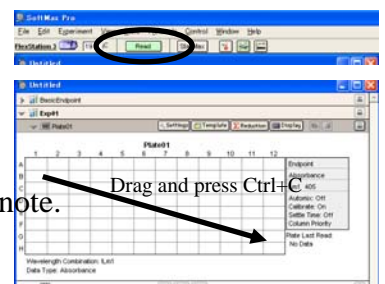
<confirm>No X mark on the device



Em-Ex>20nm
Cutoff-Ex>10nm

Choose a matching plate.
Usually, no problem by selecting the "standard".

clrbtm	Clear bottom	for absorbance
opaque	Opacity(Black)	for fluorescence
blk/clrbtm	Clear bottom (side is black)	for fluorescence



<2 wavelength measurement method (ratio)>

Add to step 6.

Wavelengths:1→2 , Lm1=Measurement excitation and emission wavelength

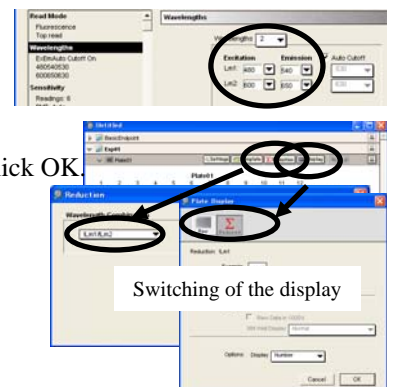
Lm2=Reference excitation and emission wavelength

Add to step 9.

Click the “Reduction” button. Select the “Lm1/Lm2” in the “Wavelength Combination” field. Click OK.

Click the “Display” button. Select “Reduced” , and click OK. (The ratio is displayed.)

Warning : Value copied to Excel is the display value.



Switching of the display

How to use luminescence mode

Corresponding plate: 6~384well plate

1. Turn on the plate reader power.

(Warning : All of the door opens and closes. Sometimes, you will hear a loud sound.)

2. Turn on the PC , and login windows.

3. Wait until the temperature is displayed. (about 3min?)

(Does not recognize the device.)

4. Double-click on the desktop of “SoftMaxPro” icon.

(Warning : The door opens. In order to close the door, press the “reading chamber” button.)

5. Click the “Setting” button in the “Plate01” tab.

【6】. Set the following items, click OK.

Read Mode : Luminescence(RLUs)

(When you check the "Read from bottom", measured from the bottom.)

Assay Plate Type : Choose the ones that you use.

Clear plate → clrbtm , White plate → opaque

7. Press “reading chamber” button. Remove the lid , and put the plate.

(If the lid is necessary, please use the seal. When you use a deep plate, please remove the spacer.)

In order to close the door, press the “reading chamber” button.

8. Start the measurement by clicking the “Read” button.

When the measurement is finished plate is ejected.

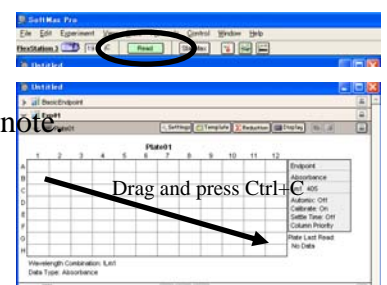
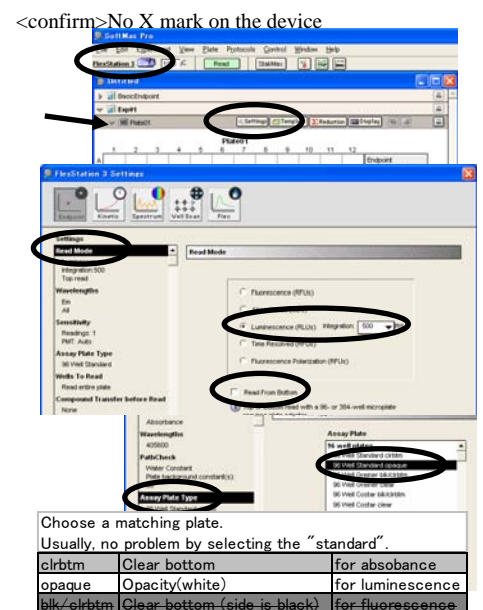
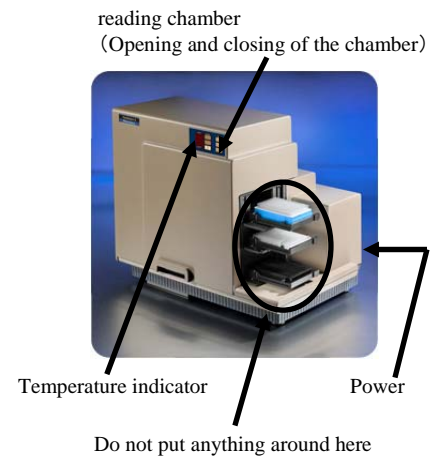
【9】. Value is displayed on the "Plate1" tab. Drag the well image , and press Ctrl+C.

10. To start the excel , and press Ctrl+V. Save the worksheet.

11. When you continue the measurement, go to step 6.

12. When the measurement is completed, exit the “SoftMaxPro” software.

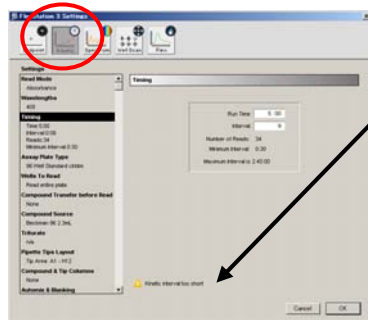
Exit the Windows, and turn off the plate reader power. Please fill in the used note.



How to use kinetic mode

Click the “kinetic” button.

The item of kinetic is added to the basic operation.



<Timing>

Run Time: Total measurement time

Interval: Measurement interval

(Set to be longer than the “Minimum Interval”.)

The minimum interval value will vary depending on the number of wells. Measurement wells are set in the “Wells to read”.

The results are displayed in a graph.

After the measurement, you can confirm all of the value in Excel.

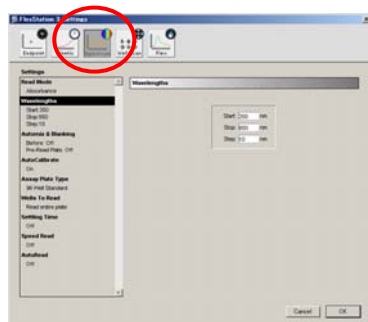
(Drag the well image , and press Ctrl+C.)

Do not use the reagent addition function in kinetic mode.

How to use spectrum mode

Click the “Spectrum” button.

The item of spectrum is added to the basic operation.



<Wavelength>

Start: start wavelength (small value)

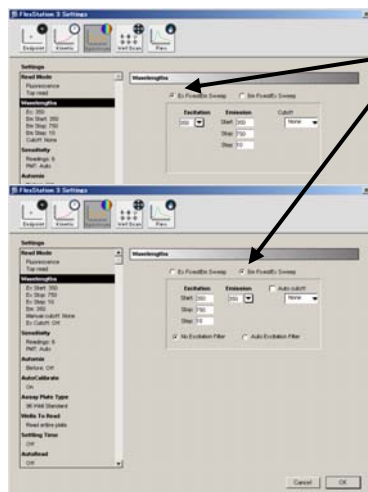
Stop: end wavelength (large value)

Step: measurement interval

The results are displayed in a graph.

After the measurement, you can confirm all of the value in Excel.

(Drag the well image , and press Ctrl+C.)



<In the case of fluorescence measurement>

You do have to fix either the excitation or emission.

In order to eliminate the excitation light, use the cut-off filter.

Copy and paste.
(all of the value pasted)

Select and double-click.
(Show graph)

Change display range

Switch Auto Y-range

Calculation

Switch Row / Calculation value

How to use Flex mode (kinetic and reagent addition)

Click “Flex” button. Item of reagent addition function and kinetic measurement will be added.

How to set the items refer to “How to use kinetic mode” and “How to use reagent addition function”.

Flex mode has the following restrictions.

Measured from the bottom. It is measured in a single column (8 samples).

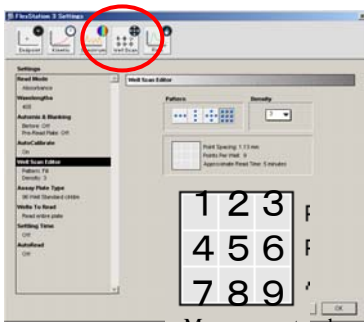
After the start of the measurement, reagent is added.



The “Time Point” is the time you want to add a reagent.
To prepare, please set to be longer than the “Minimum time”.

How to use Well Scan mode

Click “Well Scan” button. Item of well scan will be added.



Measurement order

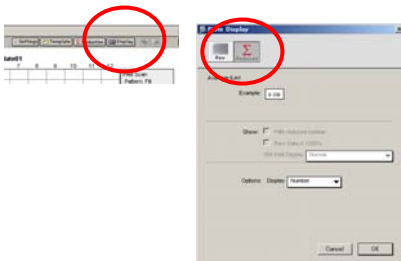
<Well Scan Editor>

Pattern : measurement pattern

Density : The number of points of one side

“Points Per Well” is total points.

The plate of large wells, it can set a large number of points.



The results are displayed in a image map.

You can confirm all of the value in Excel.

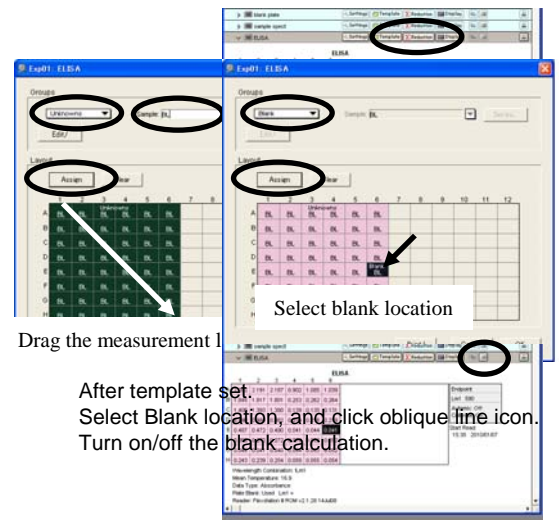
(Drag the well image , and press Ctrl+C.)

How to use Template (1 of 2)

<I want to subtract the blank>

1. Click "Template" button.
 2. Drag the well image.
 3. Set the "Groups" to "Unknown".
- Set the "Sample" to "BL". Click "Assign" button.
4. Click the blank location.
 5. Set the "Groups" to "Blank". Click "Assign" button.
 6. When you click OK, the blank is subtracted.

✘Location that is not assigned, the display will disappear.



<Quantitative method using a standard sample (ELISA)>

1. Click "Template" button.
 2. Drag the location of the first standard sample.
 3. Set the "Groups" to "Standards". Set the "Sample" to "St01".
- Input concentration value, and click "Assign" button .
- (Change of the unit can be in the edit item.)St01 is calculated as the same sample. (average value use)
4. To assign all of the standard sample.(2nd STD→St02...)
 5. To assign an unknown sample and blank.

Unknown sample : Groups=Unknowns, Sample=Un01,Un02, Un03...
Blank : Groups=Blank

✘Location that is not assigned, the display will disappear.

6. The results are displayed in standards,unknowns and standard curve tab.

Standards : Conc=setting concentration, Value=measurement value,

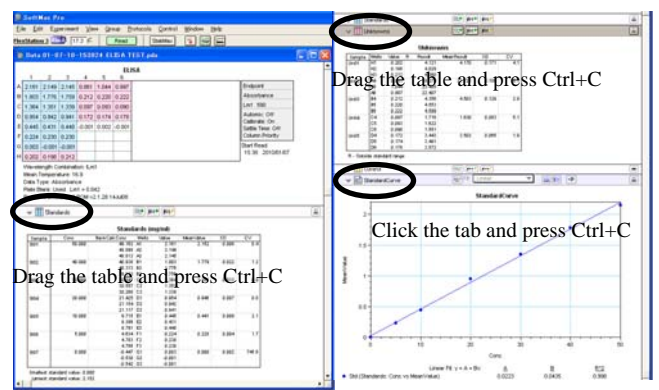
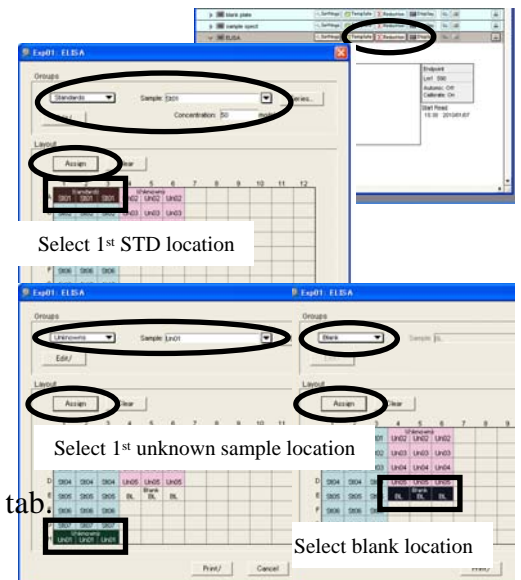
MeanValue=average value (This value is used in the calculation of the calibration curve.)

Unknowns : Value=measurement value, Result=calculated concentration, MeanResult=average concentration

Standard Curve : R^2 =Coefficient of linearity?

Standards and Unknowns tables and Standard Curve can be pasted into Excel.

(Standard Curve will change in the figure.)



How to use Template (2 of 2)

<How to use the series>

1. Click "Template" button.
2. Drag the location of all of the standard sample.
3. Click "Series" button.
4. Set the following items, click OK.
 - Start From : Pattern of concentration
 - Replicates : Number of replicate
 - Units : Unit of concentration
 - Starting value : Concentration of 1st standard sample
 - Step by : magnification
5. Drag the location of all of the unknown sample.
6. Click "Series" button.
7. Input "Start From" and "Replicate", click OK.
8. Select blank location.
9. Set the "Groups" to "Blank". Click "Assign" button.
10. Where does not have the regularity, please correct.

In the case of Top
Line up the same concentration in the transverse.
Top is 1st sample. (St01)

Enter the formula
(Examples of the double dilution)

St01=400ug/ml

St02=St01/2
=400/2=200ug/ml

St03=St02/2
=200/2=100ug/ml

If you multiply, bottom is conc.
You would think that different from the image.

Number of replicate
(The number of horizontal)

Number of replicate
(The number of horizontal)

For example...
Replicate=1

Additional Notes

You can save the settings and template information.

<How to save the protocol>

1. To create a saved condition. (Setting, Template)
2. Select “File - Save as”.
3. Change the file type to “Pro Protocol Files”. Input file name and save.
4. When you double-click the saved file, you can use.

Change the format of the data when pasted into Excel.

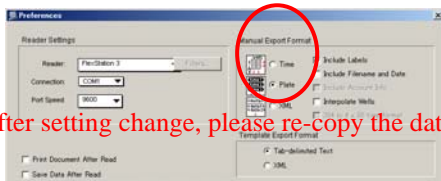
<How to change the export format>

1. Select “Edit – Preferences”.
2. Change the items in the Manual Export Format.

Time...Format for Kinetic measurement. The value of one well is arranged in a column.

Plate...Format for endpoint measurement. The value is placed in the form of a plate.

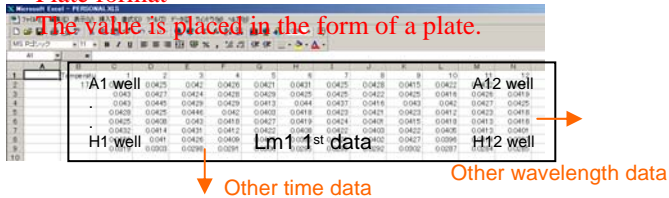
3. Click OK button. Re-copy / paste the data.



After setting change, please re-copy the data.

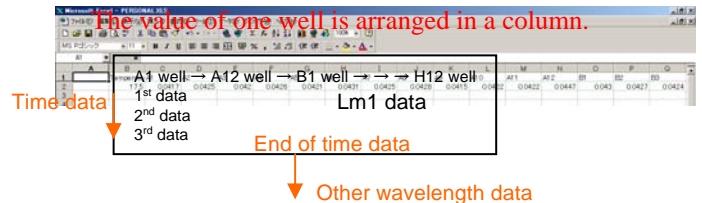
Plate format

The value is placed in the form of a plate.



Time format

The value of one well is arranged in a column.



In the case of the two-wavelength,
Lm2 data is located on the right side of Lm1 data.

In the case of the two-wavelength,
Lm2 data is located on the lower side of Lm1 data.

In the case of kinetic,
2nd data is located on the lower side of 1st data.

How to use pipetter

This manual is pipette operation only. Please refer to the other manual for the basic operation.

<How to set measurement temperature>

Press the "Temp On/Off" button on the instrument.

(Upper=measured temperature , Lower=setting temperature)

When the set value is displayed, the temperature is controlled.

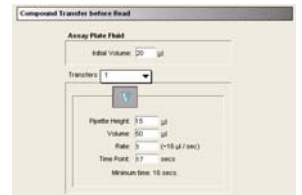
Set in the up and down buttons.

<Pipette setting>

Gray characters is not essential

Minimum volume
Flat bottom...50ul , V-bottom...20ul

Recommended material
PS or PP



Wells To Read : measurement well

Compound Transfer Before Read :The addition amount set

Initial Volume=sample volume , Transfers=The number of additional

Pipette Height=Position of addition(height 50~270ul) ,

Volume=addition volume(1~200ul) , Rate=injection speed

Time Point=injection time(Set to be larger than the Minimum Point.)



This setting
Use 4th column of the reagent.
Use the 12th column of chips.
Add the reagent to 12th column
of the sample wells.

Compound Source : Plate for the addition of reagents

Triturate : Pipetting setting(Set volume and cycle)

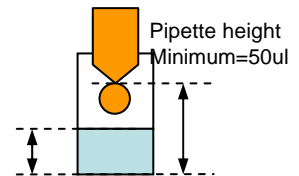
Compound & Tip Columns : Set of reagents and chip

number=Position of sample , T=Position of the tip , C=Position of the reagent

When you want to change, click on the image, select the pull-down.

Auto Mix : Time to shake the plate

Settling Time : Delay time



Initial volume

If the initial volume is less than 50ul,
the reagent will drop.

Click the OK when you are finished.

If an error is out, correct the value.

Press the "Tip rack" button on the instrument. Put the chip rack on the tray. (remove lid)

Press the "Tip rack" button again. **✘Use the specified chip. You can not re-use the chips.**

Press the "Source" button on the instrument. Put the plate containing reagents on the tray. (remove lid)

Press the "Source" button again.

How to use dual luciferase method

Add two kinds of reagent and obtain the ratio. The first reagent added is "Firefly", the next reagent is "Renila".

Start the "Soft Max pro" software.

Select the item in menu. (Protocols - Reporter Assays - Dual-Glo Luciferase)

Set the 1st measurement conditions to "PlateA_Firefly" setting.

Set the 2nd measurement conditions to "PlateB_Renila" setting.

(Renila's initial volume is the value that after the first reagent is added.)

Set chip rack, reagent plate, sample plate in the equipment.

(When adding reagents by yourself, set only sample plate.)

Click the letter of "PlateA_Firefly" and check that the bar is gray. Click read button.

(After measurement, the plate is discharged and the value of FireFly is displayed.)

Set chip rack, reagent plate, sample plate in the equipment. (for Renila measurement)

Click the letter of "PlateB_Renila" and check that the bar is gray. Click read button.

((After measurement, the plate is discharged and the value of Renila is displayed.)

If the number of samples is 4 or more, please add items by the following operation.

Click the letter of "Firefly_04" and check that the bar is gray.

Select "Edit - Duplicate "Firefly_04" from the menu. "Firefly_04Copy" is created.

Double click on "Firefly_04 Copy", change Name to "Firefly_05" and click "OK".

Repeat the operation for the required number of samples.

Open the tab of "PlateA_Firefly" and click "Template".

Click the position of the **first** sample.

Change Groups item to "**Firefly_01**". Change sample item to "**Fir01**". Click "Assign".

Click the position of the **second** sample.

Change Groups item to "**Firefly_02**". Change sample item to "**Fir01**". Click "Assign".

Set all samples with similar rules. Click "OK".

Open the tab of "PlateB_Renila" and click "Template".

Set by the same operation as Firefly. (Groups item is "Renila_***". Sample item is "Re01".)

Click "OK" when all samples are set.

When you open the "Firefly_01" tab, the values of "Firefly_01" and "Renila_01" are displayed.

Likewise, when you open the "Firefly_02" tab, the values of "Firefly_02" and "Renila_02" are displayed.

FireflyRLU=Firefly's value , AvgFF=Average of Firefly's value

RenRLU=Renila's value , AvgRen=Average of Renila's value

NormFF=Ratio of Firefly to Renila , AvgNormFF=Average of ratio

SDnormFF=Standard deviation of ratio

<How to save the data>

Select "File - Import/Export – Export" from menu.

Check "Plate Section", "Group Section". Specify the location and save(Click "保存" button.).

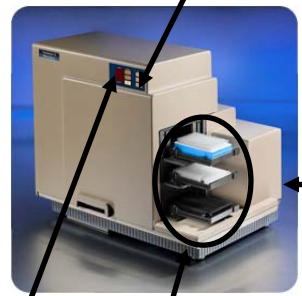
How to use time-resolved fluorescence mode

Wavelength range:250nm~850nm , (Max) 4 point simultaneous measurement

Corresponding plate:6~384well plate

Minimum delay time:50us Dual TR-F not support.

reading chamber
(Opening and closing of the chamber)



Temperature indicator

Power

Do not put anything around here

1. Turn on the plate reader power.

(Warning : All of the door opens and closes. Sometimes, you will hear a loud sound.)

2. Turn on the PC , and login windows.

3. Wait until the temperature is displayed. (about 3min?)

(Does not recognize the device.)

4. Double-click on the desktop of “SoftMaxPro” icon.

(Warning : The door opens. In order to close the door, press the “reading chamber” button.)

5. Click the “Setting” button in the “Plate01” tab.

【6】. Set the following items, click OK.

Read Mode : Time Resolved(RFUs)

Choose delay time (Integ delay) and integration time (Integration).

(When you check the "Read from bottom", measured from the bottom.)

Wavelengths : Enter excitation and emission wavelength.

Assay Plate Type : Choose the ones that you use.

Clear plate→clrbtm , Black plate→opaque

7. Press “reading chamber” button. Remove the lid , and put the plate.

(If the lid is necessary, please use the seal. When you use a deep plate, please remove the spacer.)

In order to close the door, press the “reading chamber” button.

8. Start the measurement by clicking the “Read” button.

When the measurement is finished plate is ejected.

【9】. Value is displayed on the "Plate1" tab.

Drag the well image , and press Ctrl+C.

10. To start the excel , and press Ctrl+V. Save the worksheet.

11. When you continue the measurement, go to step 6.

12. When the measurement is completed, exit the “SoftMaxPro” software.

Exit the Windows, and turn off the plate reader power.

Please fill in the used note.

<confirm>No X mark on the device

Choose a matching plate.		
Usually, no problem by selecting the "standard".		
clrbtm	Clear bottom	for absorbance
opaque	Opacity(Black)	for fluorescence
blk/clrbtm	Clear bottom (side is black)	for fluorescence

How to use fluorescence polarization mode

Wavelength range:250nm~850nm , (Max) 4 point simultaneous measurement

Corresponding plate:6~384well plate

1. Turn on the plate reader power.

(Warning : All of the door opens and closes. Sometimes, you will hear a loud sound.)

2. Turn on the PC , and login windows.

3. Wait until the temperature is displayed. (about 3min?)

(Does not recognize the device.)

4. Double-click on the desktop of “SoftMaxPro” icon.

(Warning : The door opens. In order to close the door, press the “reading chamber” button.)

5. Click the “Setting” button in the “Plate01” tab.

【6】. Set the following items, click OK.

Read Mode : Fluorescence Polarization (RFUs)

Wavelengths : Enter excitation and emission wavelength.

Assay Plate Type : Choose the ones that you use.

Clear plate→clrbtm , Black plate→opaque

Em-Ex>20nm
Cutoff-Ex>10nm

7. Press “reading chamber” button. Remove the lid , and put the plate.

(If the lid is necessary, please use the seal. When you use a deep plate, please remove the spacer.)

In order to close the door, press the “reading chamber” button.

8. Start the measurement by clicking the “Read” button.

When the measurement is finished plate is ejected.

【9】. Value is displayed on the "Plate1" tab.

Drag the well image , and press Ctrl+C.

10. To start the excel , and press Ctrl+V. Save the worksheet.

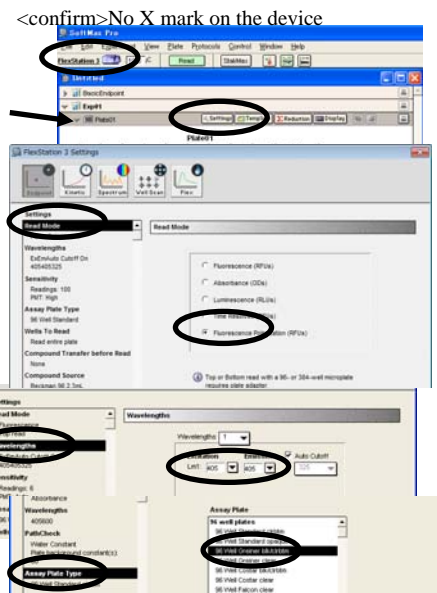
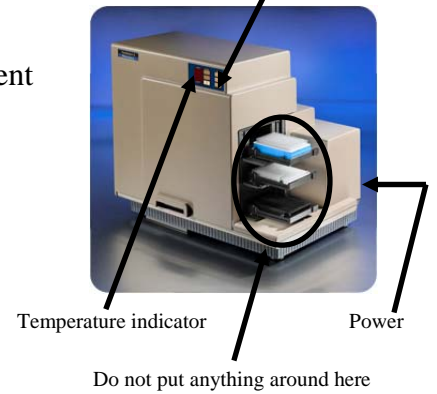
11. When you continue the measurement, go to step 6.

12. When the measurement is completed, exit the “SoftMaxPro” software.

Exit the Windows, and turn off the plate reader power.

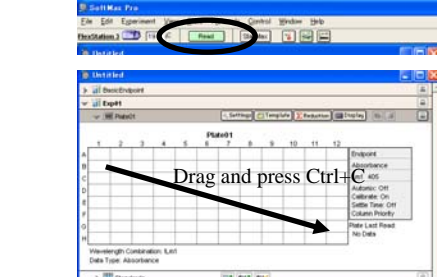
Please fill in the used note.

reading chamber
(Opening and closing of the chamber)



Choose a matching plate.
Usually, no problem by selecting the “standard”.

clrbtm	Clear bottom	for absorbance
opaque	Opacity(Black)	for fluorescence
blk/clrbtm	Clear bottom (side is black)	for fluorescence



<How to change display>

Click the “Display” button on the “Plate1” tab. Select “Raw”.

In the "FP Display" item, select what you want to display. Initial display is “Polarization (mP)”.

(When copying to Excel, the displayed value will be copied.)

(When Raw is displayed, S value and P value are copied.)

$$\text{Polarization (mP)} = 1000 * (P - (G * S)) / (P + (G * S))$$

$$\text{Anisotropy (r)} = (P - (G * S)) / (P + (2G * S))$$

