



# **AGILECON SOFTWARE**

## **USER MANUAL**

Version 2.0

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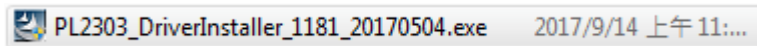
## **System Requirements**

- CPU Pentium 4 2.0GHz above for Windows 10 or above.
- 2GB of RAM or above for Windows 10 or above.
- Microsoft .NET Framework 3.5
- 50MB of available hard drive for the program files
- CD ROM drive
- 16bit color display with pixel resolution 1280 x 768 or above.
- Keyboard, Mouse, and RS232 serial port or USB port



## Software Installation

To install AgileCon\_2.0

1. Start Windows and close all unnecessary Windows applications.
2. Insert the software CD into the CD-ROM drive. The installer user interface is displayed.
3. Click on the "PL2303\_DriverInstaller\_1181\_20170504" to install USB driver.



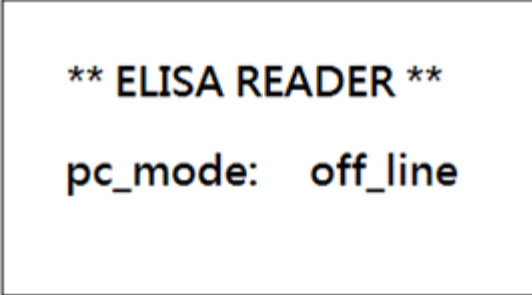
4. Follow the on-screen instruction.
5. After the installation is completed, click "Finish".
6. Click on the "setup.exe" to setup AgileCon\_2.0 software

Name	Date modified	Type
 AgileCon 2.0_v2.0.19	4/10/2018 2:21 PM	Windows Installer ...
 setup	4/10/2018 2:21 PM	Application

7. Follow the on-screen instruction.
8. After the installation is completed, click "Finish".

## **AGILE READER Instrument Setup**

1. Be sure the AGILE READER instrument is in standalone mode.  
Method to switch between standalone and PC modes on AGILE READER:  
Turn off the instrument first, then press the “OPTION” key while turn on the instrument again, it will switch to other mode.
2. On AGILE READER standalone mode, please go to SETUP / COMPUTER.
3. Move “UP/DOWN” buttons to select USB port and press “ENTER” to confirm selection with mark “S” shown on the right side of that port.
4. Power off and power on the AGILE READER again, then press the “OPTION” key while turn on the instrument, it will switch to PC mode.
5. The instrument starts to do initialization.
6. After initialized, be sure the screen show “pc\_mode” as figure 1 below.



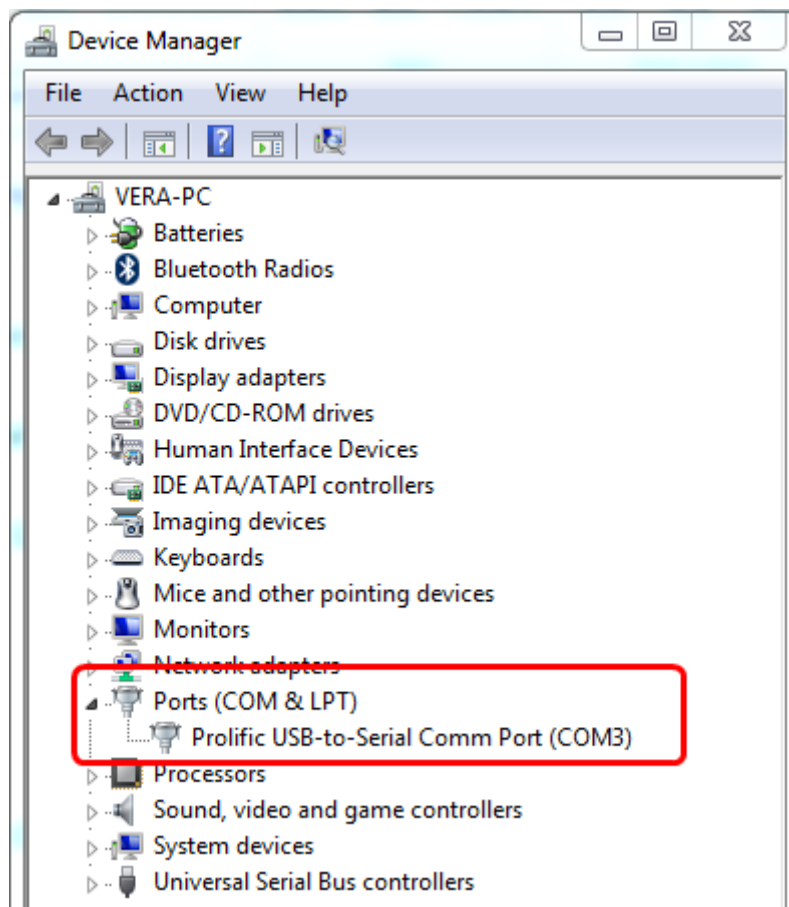
```
** ELISA READER **  
pc_mode:  off_line
```

**Figure 1**

## To Start AgileCon\_2.0

Connect the PC and the instrument with an USB cable, then power meter up. Operate your Windows system as the following steps.

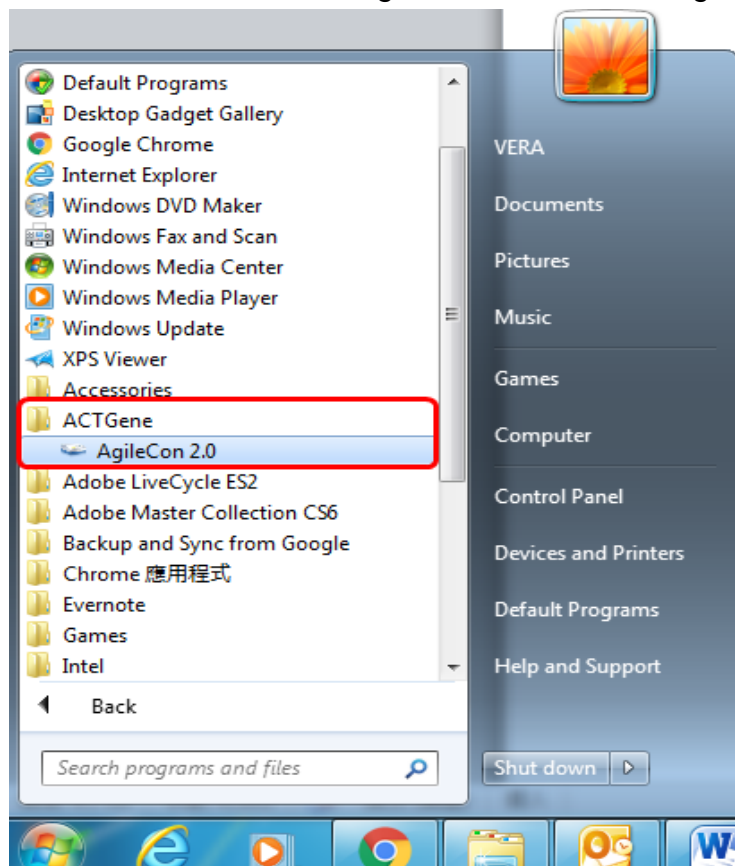
1. Press Start menu / Control Panel / Device Manager / Ports (COM & LPT)
2. Find and memorize the “Prolific USB-to-Serial Comm Port” number(COM\_number).





3. Go to AgileCon\_2.0.

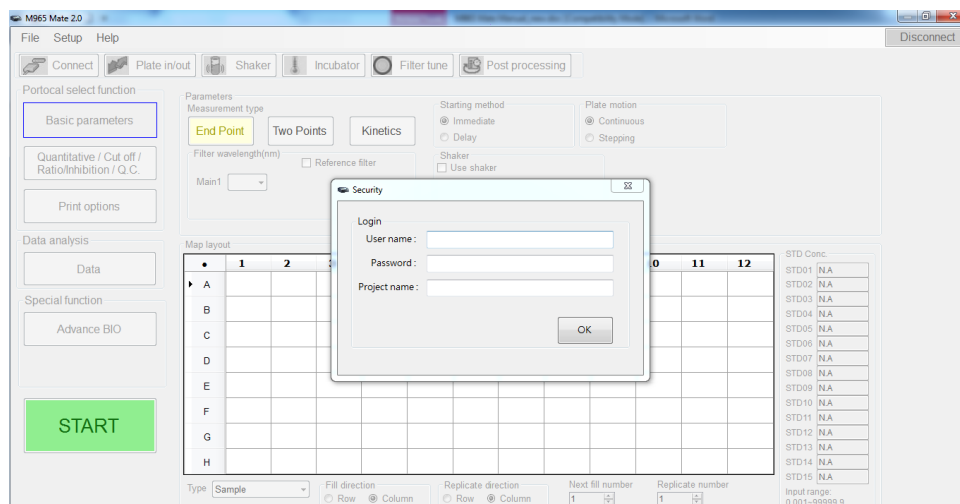
From Start menu → All Programs → ACTGene → AgileCon\_2.0



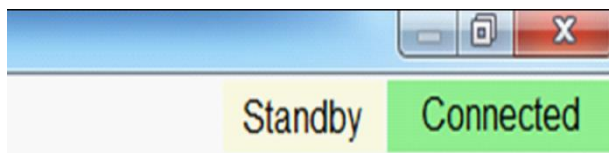
4. For the first time login, key in default value "admin" for both User name and Password in Security window below. Press OK to start comport connection.

User name : **admin**

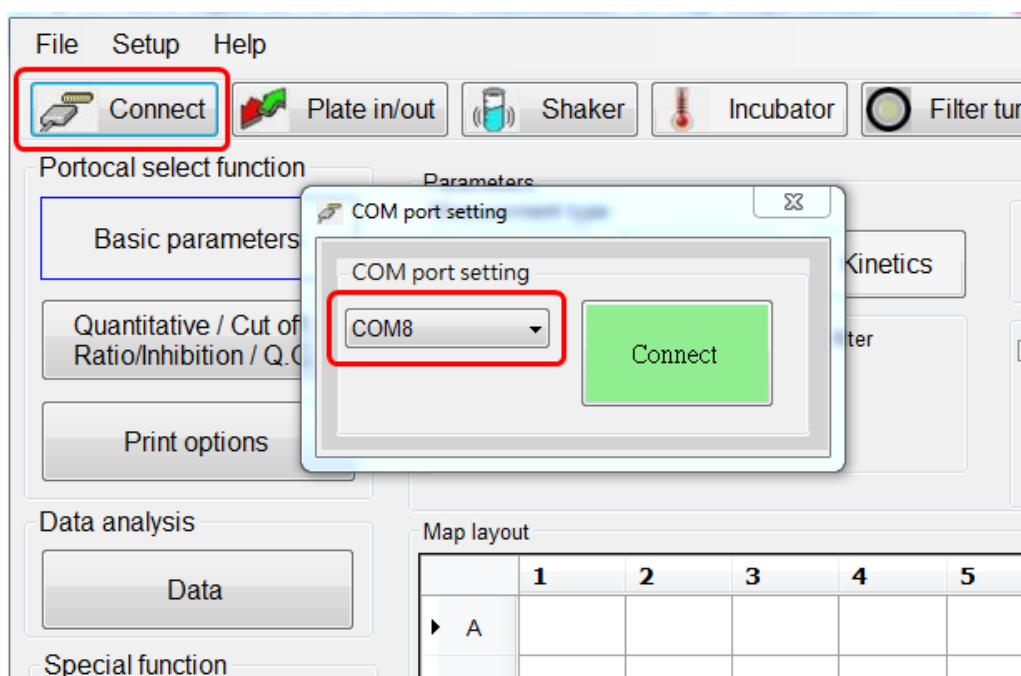
Password : **admin**



5. If PC is successfully connected to AGILE READER, the "Connected" sign with green background will appear on the upper right message area of the screen.



6. In case PC cannot connect to the instrument, please press the Connect icon to select corresponding COM port as shown in step 2. and then press Connect button to confirm selection



7. Now, the instrument and AgileCon software are ready to perform the experiment.

# AgileCon\_2.0 Menu Software Structure

## Main Window Overview

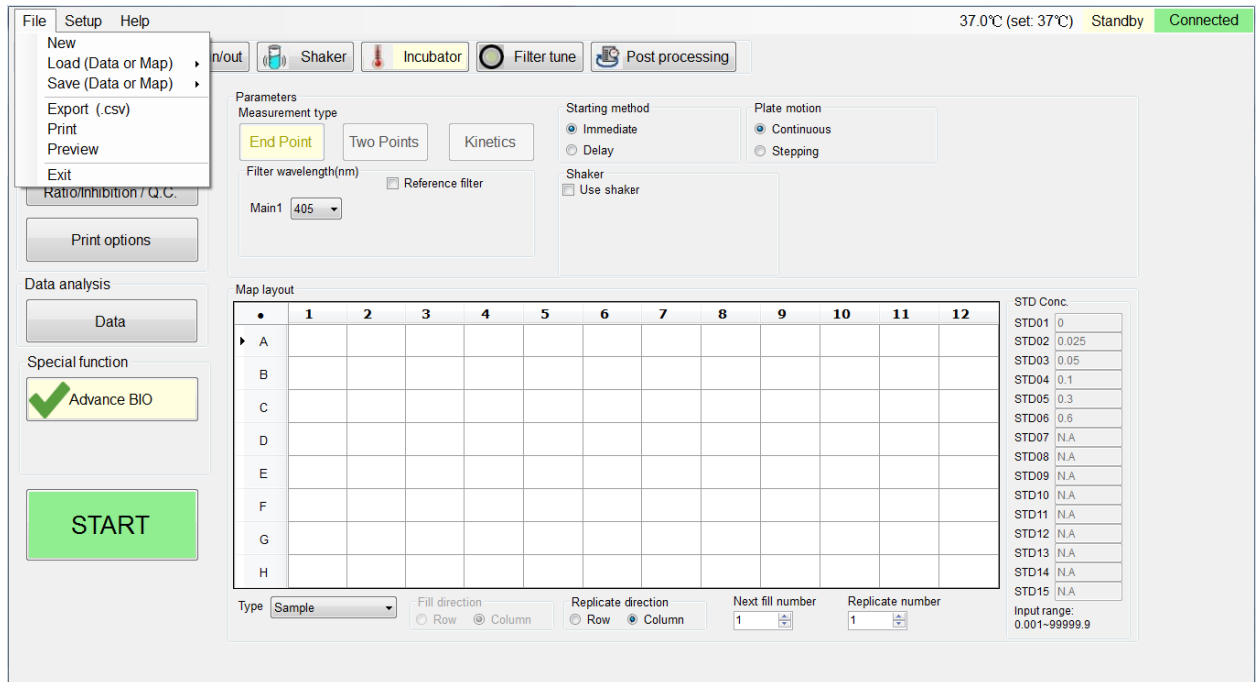
- Section A: Menu
- Section B: Tool bar
- Section C: Message
- Section D: Temperature monitor
- Section E: Working area
- Section F: Data review
- Section G: Special function

The screenshot shows the AgileCon\_2.0 software interface. The top menu bar (A) includes File, Setup, and Help. The tool bar (B) contains icons for Connect, Plate in/out, Shaker, Incubator, Filter tune, and Post processing. The status bar (C) shows the temperature as 37.0°C (set: 37°C) and the system status as Standby and Connected. The main working area (E) is divided into several sections: Portocal select function (Basic parameters, Quantitative / Cut off / Ratio/Inhibition / Q.C., Print options), Parameters (Measurement type: End Point, Two Points, Kinetics; Filter wavelength: 405 nm; Starting method: Immediate, Delay; Plate motion: Continuous, Stepping; Shaker: Use shaker), and Map layout (an 8x12 grid). The data review section (F) includes a Data button. The special function section (G) includes an Advance BIO button with a green checkmark. A large green START button is located at the bottom left. The right side of the interface displays a list of STD Conc. values for wells STD01 through STD15, with STD01-05 having numerical values and STD06-15 having 'N.A.'. Below the grid, there are controls for Type (Sample), Fill direction (Row, Column), Replicate direction (Row, Column), Next fill number (1), and Replicate number (1). The input range is specified as 0.001-99999.9.

## Section A Menu

### File Menu

The File Menu contains the file and print functions for the experiment data and mapping file.



**New:** Create a new experiment

**Load (Data or Map):** Load a stored experiment, results or map layout

**Save (Data or Map):** Save the experiment parameters, results or map layout

**Export (.csv):** Export report to a file with ".csv" file extension

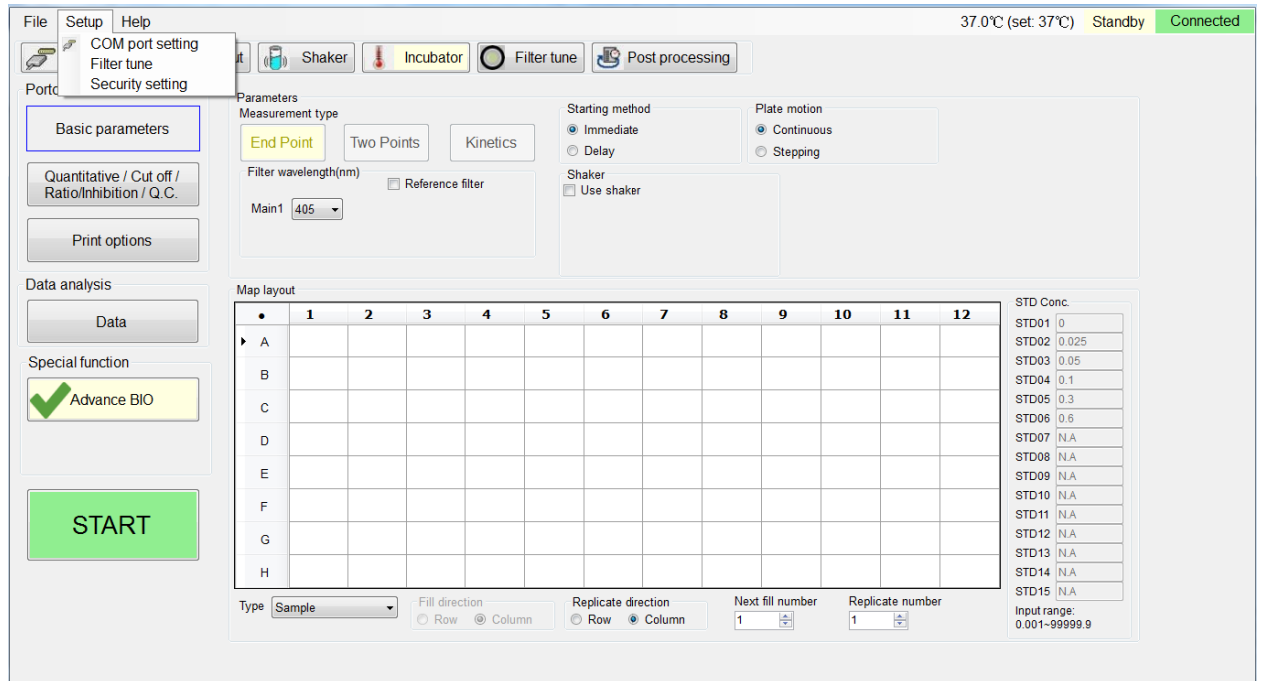
**Print report:** Select experiment to print out

**Preview:** Preview experiment report format

**Exit:** Close the AgileCon\_2.0 software

## Setup Menu

The setup menu contains the AgileCon\_2.0 system configuration and user account management.



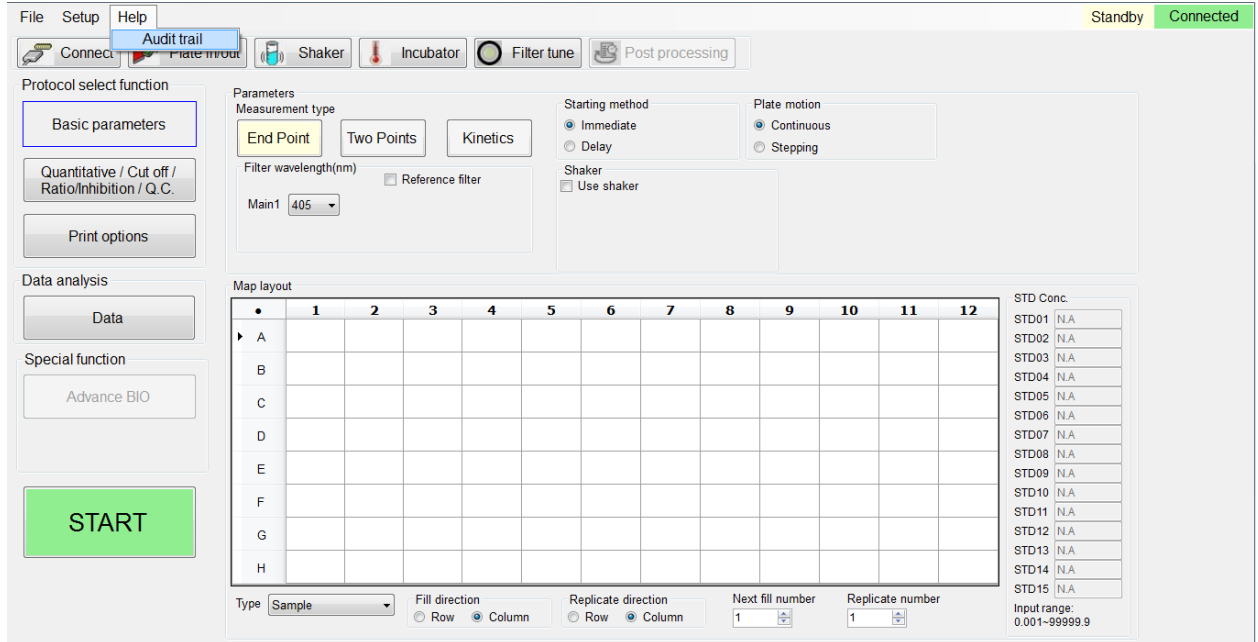
**COM port setting:** To set the communication COM port between the instrument and PC.

**Filter tune:** The AgileCon\_2.0 can setup 8 different wavelengths at most by inserting the corresponding filters to meter filter wheel. Configure the filter wavelengths according to the inserted filters.

**Security setting:** To create a new user ID and set up the security level, or delete the user ID.

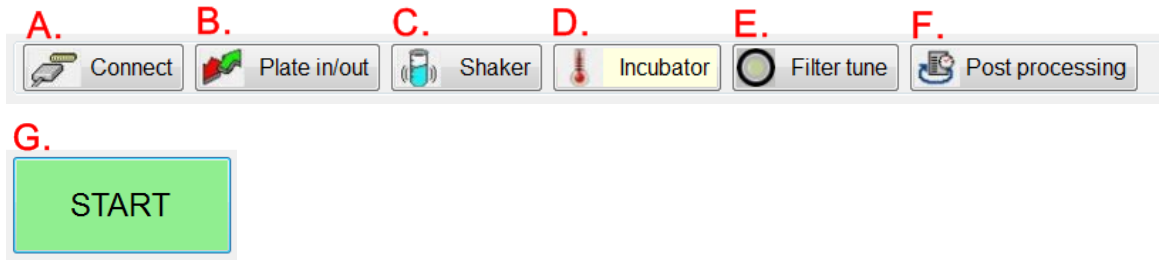
## Help Menu

The help menu provides information on software version, contact information of the vender, and the user activity records.



**Audit trail:** To record the user activity for trailing.

## Section B Toolbar



**Connect:** To set the COM port to communicate between instrument and the PC

**Plate in/out:** To open or close the plate compartment

**Shaker:** To shake the plate with desired speed and shaking time

**Incubator:** To control the incubator with desired temperature, and display temperature reading on section D temperature monitor.

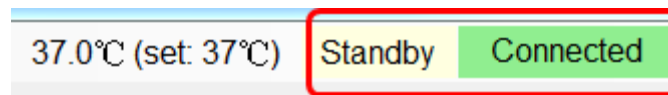
**Filter tune:** To set up the wavelengths of installed filters on 8-slot filter wheel, and have the meter tune the light intensity for each filter.

**Post processing:** Use the current protocol to re-process data results

**Start:** To start the experiment with current protocol

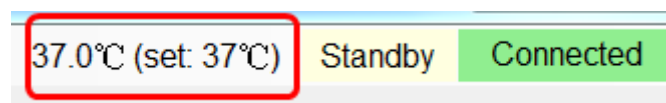
## Section C Message

During operation, the current status will be shown on the upper right of the screen.



## Section D Temperature monitor

When the incubator is activated, the set and actual temperatures are shown on the left of the message area.



## Section E Working area & F Data review

The AgileCon\_2.0 allows you to define measurement protocols and analyze obtained microplate data. The protocol parameters are input in E Working area, and the test data is shown in F Data review.

The screenshot shows the AgileCon\_2.0 software interface. The top bar includes 'File Setup Help' and '37.0°C (set: 37°C) Standby Connected'. Below the top bar are icons for 'Connect', 'Plate in/out', 'Shaker', 'Incubator', 'Filter tune', and 'Post processing'. The main interface is divided into several sections:

- Portocal select function:** Includes 'Basic parameters', 'Quantitative / Cut off / Ratio/Inhibition / Q.C.', and 'Print options'.
- Parameters:** Includes 'Measurement type' (End Point, Two Points, Kinetics), 'Starting method' (Immediate, Delay), 'Plate motion' (Continuous, Stepping), 'Filter wavelength(nm)' (Main1: 405), and 'Shaker' (Use shaker).
- Map layout:** A grid showing rows A-H and columns 1-12. The 'E. Working area' label is overlaid on this grid.
- Data analysis:** Includes 'Data review' (highlighted in red) and 'Data'.
- Special function:** Includes 'Advance BIO' (checked).
- START:** A large green button.

On the right side, there is a list of 'STD Conc.' values for STD01 through STD15, and an 'Input range' of 0.001-99999.9.

## Section G Special function

The special function is customized for the Biotest reagent test. This experiment effects only in conjunction with the Biotest reagents.

The screenshot shows the AgileCon\_2.0 software interface, similar to the previous one, but with the 'Special function' section highlighted in red. The 'Special function' section includes a checked 'Advance BIO' option. The 'Data analysis' section is also visible, with 'Data review' and 'Data' options. The 'Map layout' grid and other parameters are the same as in the previous screenshot.

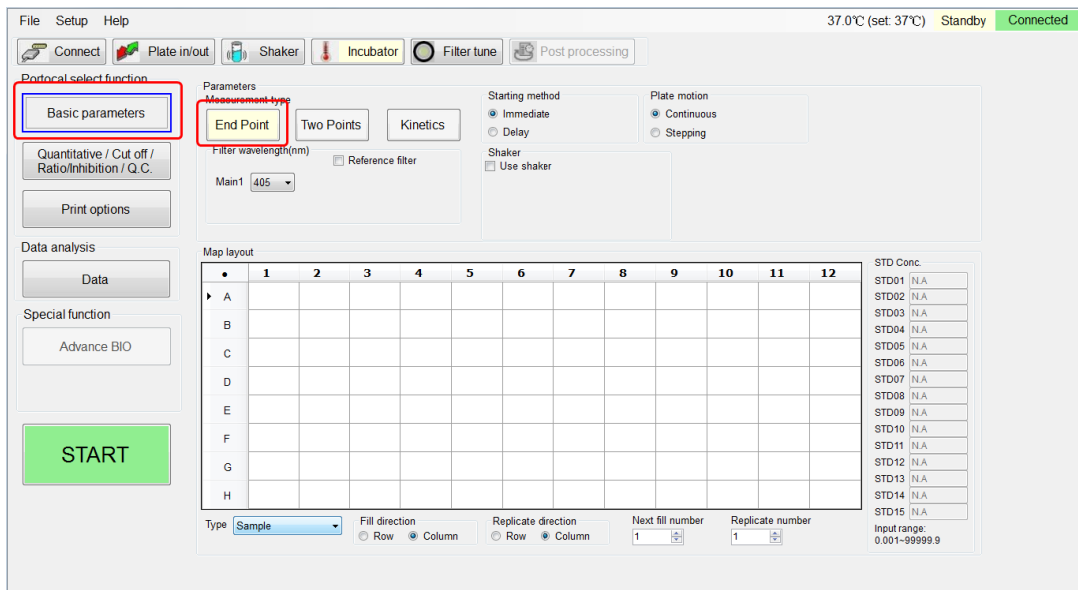


## AgileCon\_2.0 Function

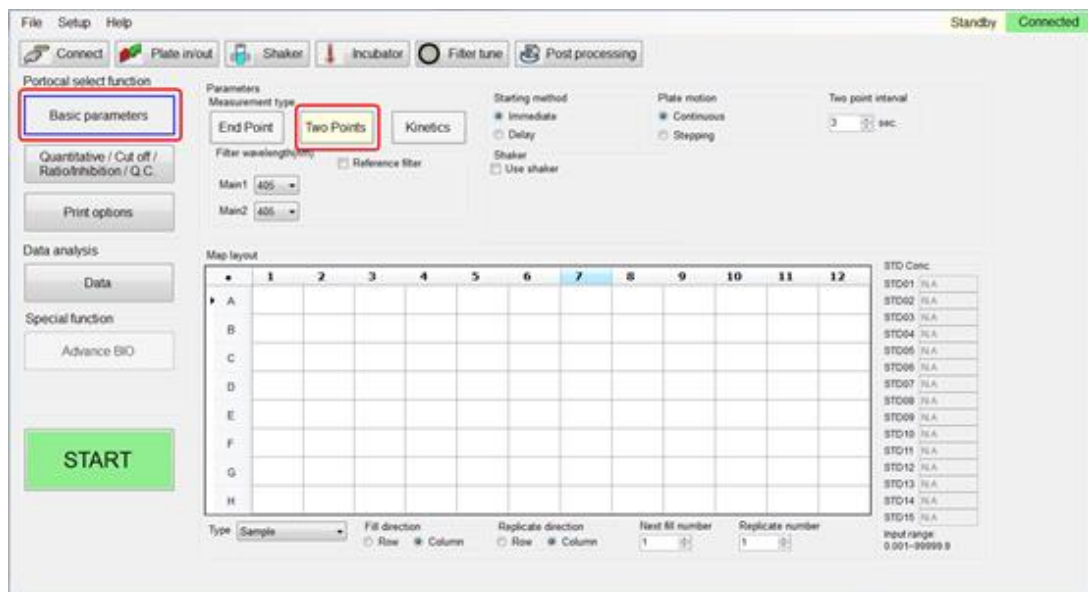
### Basic Parameters

**Measurement types:** The AgileCon\_2.0 provides three types of measurement, i.e. End point, Two point, and Kinetic measurement.

**End point read:** During the end point read, the AGILE READER reads at one wavelength, with one-reference wavelength read as optional

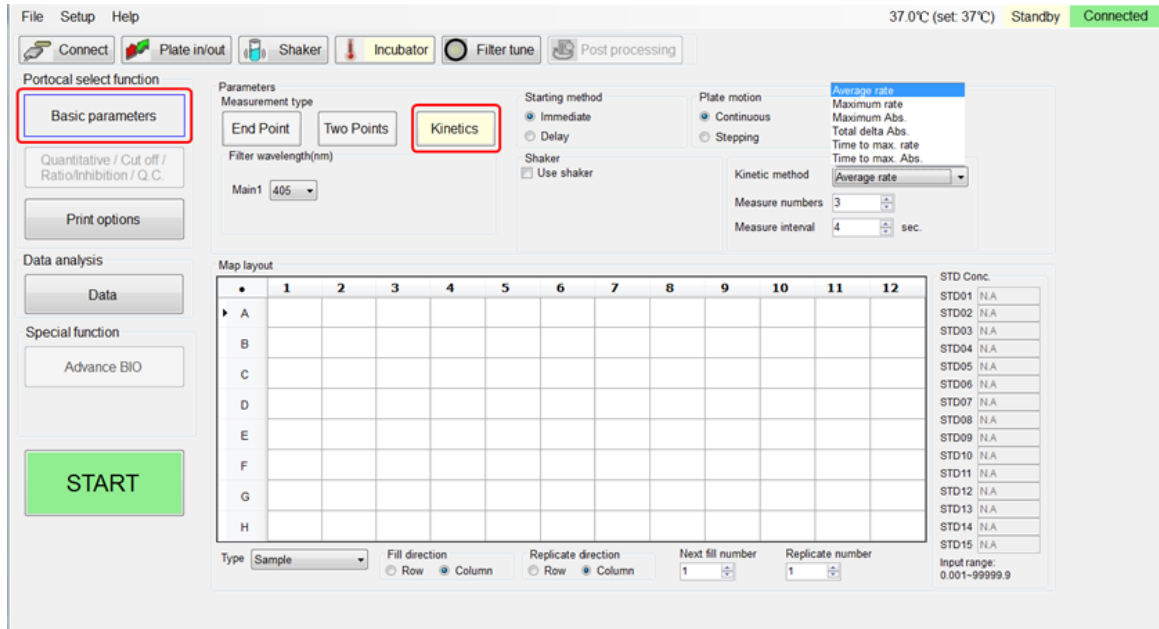


**Two points read:** During the two points read, the AGILE READER reads at two wavelengths, with two-reference wavelength read as



optional.

**Kinetics read:** During the kinetics read, users can define the kinetic method by selecting Average rate, Maximum rate, Maximum Abs, Total



delta Abs, Time to max rate, or Time to max Abs in the Kinetic method

list. The user can also define the measure numbers and interval.

To Set up a measurement with End point, Two points or Kinetic method, user need to define following parameters.

### Primary and Reference wavelengths

If a Primary wavelength is defined alone, the AGILE READER reads the plate only once at a single wavelength. If a Reference wavelength is defined, the plate will be read twice and automatically calculate the delta Abs between these two readings.

Method to set up the Primary and Reference wavelengths:

1. Select the Measurement type of End Point, or Two Points.
2. Enter the Primary wavelength in Main1 or Main2, and the reference Ref1 or Ref2

### Starting method to read plates

If the "Immediate" option is selected, the instrument starts reading the plate right after the Start button is pressed. Users can also define the

period of the plate reading delay.

To define the starting method,

1. Select the "Immediate" option.
2. Or choose "Delay", then input the delay time in second.

### **Plate motion**

Users can select the plate motion as stepping in milliseconds or continuous mode.

### **The built-in Incubator**

The incubator will keep the plate stay at temperature-controlled environment.

Users can activate the incubator by

1. Clicking the incubator button on toolbar to display the incubator pop-up menu.
2. Entering the desired temperature on the pop-up menu, and press "Activate" tab to start the temperature control.

### **The built-in Shaker**

The built-in Shaker in the instrument allow user to define speed setting as Low 8Hz, Medium 11Hz, or High 14Hz. Users can also define the shaking period.

To enable the shaker,

1. Click the shaker button
2. Select the speed to be Low, Medium or High
3. Define the shaking period in second.

## Well Mapping

Users can define five types of different wells. They are Blank, Standard, Sample, Positive, and Negative on the Type menu at Map layout.

The screenshot displays the software interface for well mapping. The 'Map layout' section is highlighted with a red box. It shows a 6x12 grid of wells with the following identifiers:

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK01 Z01-1				STD01 C01-1	STD02 C02-1	STD03 C03-1	STD04 C04-1	STD05 C05-1	STD06 C06-1		
B								SAM03 T03-1	SAM10 T10-1	SAM15 T15-1		
C								SAM04 T04-1	SAM11 T11-1	SAM16 T16-1		
D								SAM05 T05-1	SAM12 T12-1	SAM17 T17-1		
E								SAM06 T06-1	SAM13 T13-1	SAM18 T18-1		
F								SAM07 T07-1	POS01 P01-1	SAM19 T19-1		
								SAM08 T08-1	NEG01 N01-1	SAM20 T20-1		
								SAM01 T01-1	SAM01 T01-2	SAM01 T01-3		

The 'Type' dropdown menu is open, showing the following options: Blank, Positive, Negative, Sample, and Standard. The 'Sample' option is selected.

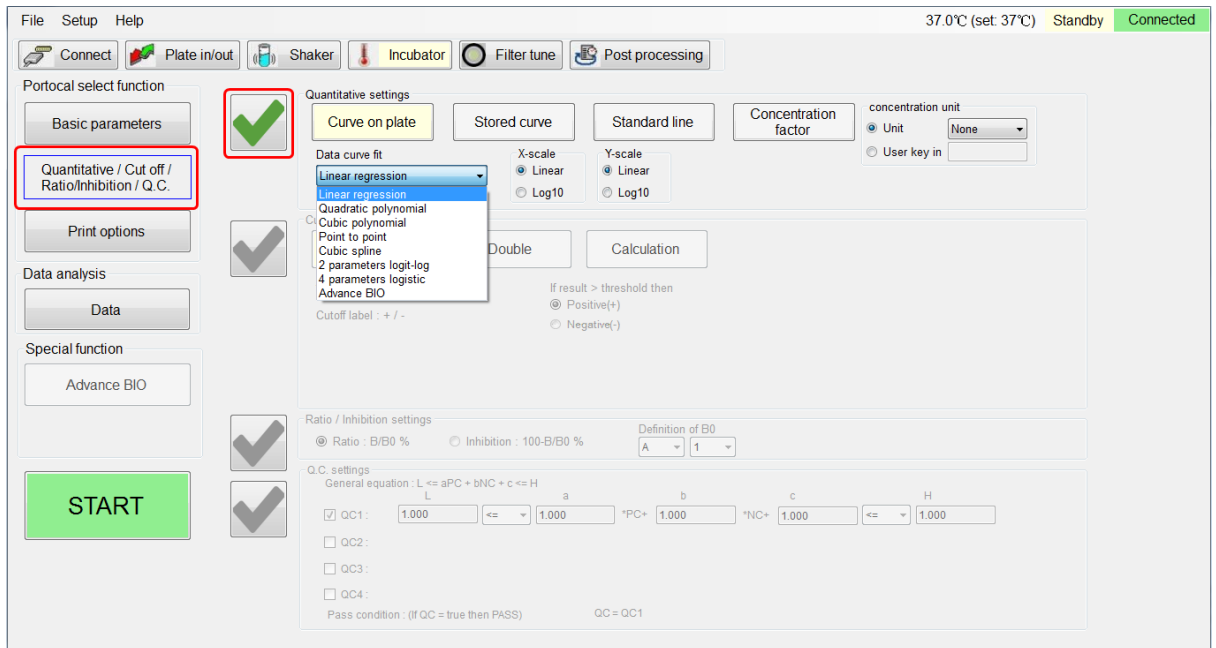
Other interface elements include: File, Setup, Help; Connect, Plate in/out, Shaker, Incubator, Filter tune, Post processing; Portocal select function; Parameters (Measurement type: End Point, Two Points, Kinetics; Starting method: Immediate, Delay; Plate motion: Continuous, Stepping; Filter wavelength: 405 nm; Reference filter); Data analysis; Special function; and a large green START button.

## Quantitative / Cut off / Ratio / Inhibition / QC

### Quantitative setting

The AgileCon\_2.0 allows user to define quantitative analysis to determine the sample concentration. Seven types of curve fitting equations are built to calculate standard polynomial coefficients. Users can select Curve on plate, Stored curve, Standard line, or Concentration factor to define Quantitative method.

1. Click the Quantitative / Cut off / Ratio / Inhibition / QC button.
2. Click the check mark in front of the Quantitative settings area, and be sure the check mark turned into green.
3. Define the desired parameters.



## Cutoffs

Cutoffs are used to classify results. Users can define three Cutoff methods as Single, Double or Calculation.

1. Click the Quantitative / Cut off / Ratio / Inhibition / QC button.
2. Click the check mark in front of the Cutoff settings area, and be sure the check mark turned to green.
3. Define the desired parameters.

The screenshot displays the software's main control panel. At the top, there is a menu bar with 'File', 'Setup', and 'Help'. On the right, the temperature is set to 37.0°C (set: 37°C) and the status is 'Standby' and 'Connected'. Below the menu bar are several function buttons: 'Connect', 'Plate in/out', 'Shaker', 'Incubator', 'Filter tune', and 'Post processing'. The left sidebar contains a 'Portocal select function' section with buttons for 'Basic parameters', 'Quantitative / Cut off / Ratio/Inhibition / Q.C.' (highlighted with a red box), 'Print options', 'Data analysis' (with a 'Data' button), and 'Special function' (with an 'Advance BIO' button). A large green 'START' button is at the bottom left. The main area is divided into several settings sections: 'Quantitative settings' with buttons for 'Curve on plate', 'Stored curve', 'Standard line', and 'Concentration factor'; 'Cutoff settings' with buttons for 'Single', 'Double', and 'Calculation' (highlighted with a green checkmark in a red box), and a 'Threshold' field set to 0; 'Ratio / Inhibition settings' with radio buttons for 'Ratio : B/B0 %' and 'Inhibition : 100-B/B0 %', and a 'Definition of B0' dropdown set to 'A' and '1'; and 'Q.C. settings' with a general equation  $L \leq aPC + bNC + c \leq H$  and four QC checkboxes (QC1 checked, QC2, QC3, QC4 unchecked). The 'Pass condition' is '(If QC = true then PASS)' and 'QC = QC1'.

## Ratio/Inhibition

The AgileCon\_2.0 will take a reference (B0) and other samples (B) to calculate the Ratio/Inhibition

1. Click the Quantitative / Cut off / Ratio / Inhibition / QC button.
2. Click the check mark in form of the Ratio/Inhibition settings area, and be sure the check mark turned green.
3. Define the desired parameters.

The screenshot displays the AgileCon\_2.0 software interface. At the top, there is a status bar showing '37.0°C (set: 37°C)', 'Standby', and 'Connected'. Below this is a menu bar with 'File', 'Setup', and 'Help'. A toolbar contains icons for 'Connect', 'Plate in/out', 'Shaker', 'Incubator', 'Filter tune', and 'Post processing'. The main interface is divided into several sections:

- Portocal select function:** Includes buttons for 'Basic parameters', 'Quantitative / Cut off / Ratio/Inhibition / Q.C.' (highlighted with a red box), 'Print options', 'Data analysis' (with a 'Data' button), and 'Special function' (with an 'Advance BIO' button). Each button has a corresponding checkmark icon to its right.
- Quantitative settings:** Features buttons for 'Curve on plate', 'Stored curve' (highlighted in yellow), 'Standard line', and 'Concentration factor'. It also includes a 'File name' field and a 'concentration unit' dropdown menu with options for 'Unit' and 'User key in'.
- Cutoff settings:** Includes buttons for 'Single', 'Double', and 'Calculation'. It has a 'Threshold' field set to '0' and a section for 'If result > threshold then' with radio buttons for 'Positive(+)' (selected) and 'Negative(-)'. A 'Cutoff label : + / -' field is also present.
- Ratio / Inhibition settings:** Features radio buttons for 'Ratio : B/B0 %' (selected) and 'Inhibition : 100-B/B0 %'. It includes a 'Definition of B0' dropdown menu with options 'A' and '1'.
- Q.C. settings:** Displays a 'General equation :  $L \leq aPC + bNC + c \leq H$ '. Below this are checkboxes for 'QC1', 'QC2', 'QC3', and 'QC4'. The 'QC1' checkbox is checked. The 'Pass condition : (if QC = true then PASS)' and 'QC = QC1' are also shown.

A large green 'START' button is located at the bottom left of the interface.

## Q.C.

The AgileCon\_2.0 provides Q.C. algorithm for experiment to determine the results.

1. Click the Quantitative / Cut off / Ratio / Inhibition / QC button.
2. Click the check mark in front of the Q.C. settings area, and be sure the check mark turned green.
3. Define the desired parameters.

File Setup Help 37.0°C (set: 37°C) Standby Connected

Connect Plate in/out Shaker Incubator Filter tune Post processing

Portocal select function

Basic parameters

Quantitative / Cut off / Ratio/Inhibition / Q.C.

Print options

Data analysis

Data

Special function

Advance BIO

START

Quantitative settings

Curve on plate Stored curve Standard line Concentration factor

concentration unit

Unit None

User key in

File name :

Cutoff settings

Single Double Calculation

Threshold : 0

If result > threshold then

Positive(+)

Negative(-)

Cutoff label : + / -

Ratio / Inhibition settings

Ratio : B/B0 % Inhibition : 100-B/B0 %

Definition of B0 A 1

Q.C. settings

General equation :  $L \leq aPC + bNC + c \leq H$

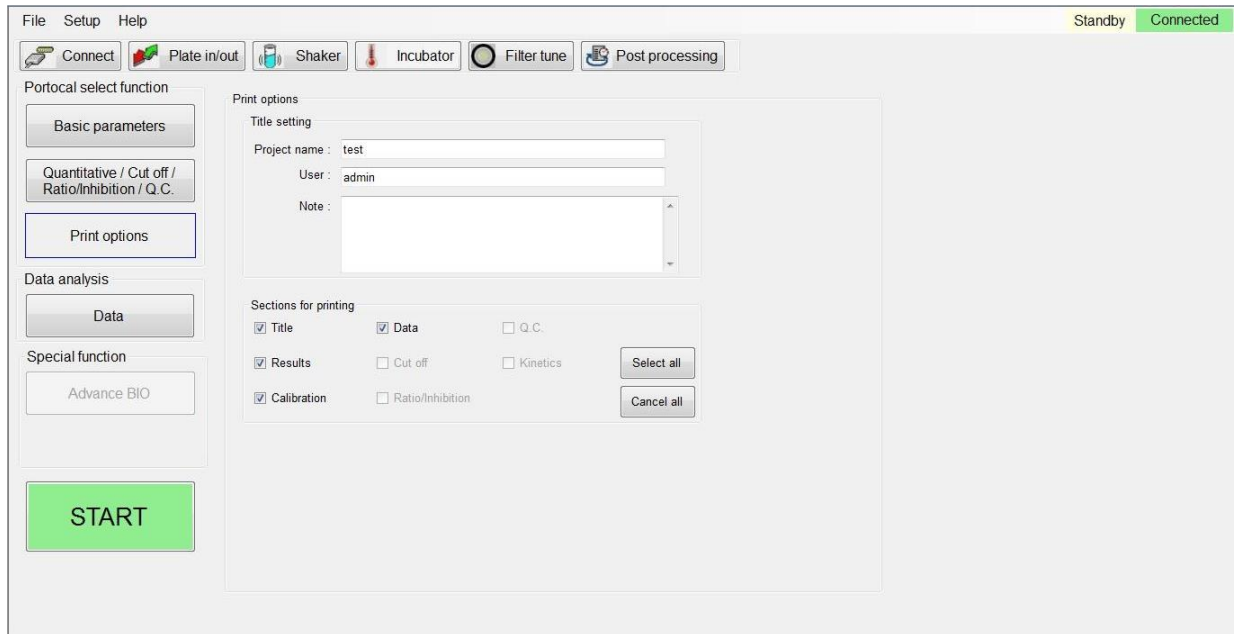
	L		a		b		c		H
<input checked="" type="checkbox"/> QC1 :	1.000	<=	1.000	*PC+	1.000	*NC+	1.000	<=	1.000
<input checked="" type="checkbox"/> QC2 :	1.000	<=	1.000	*PC+	1.000	*NC+	1.000	<=	1.000
<input checked="" type="checkbox"/> QC3 :	1.000	<=	1.000	*PC+	1.000	*NC+	1.000	<=	1.000
<input checked="" type="checkbox"/> QC4 :	1.000	<=	1.000	*PC+	1.000	*NC+	1.000	<=	1.000

Pass condition : (if QC = true then PASS) QC = QC1 AND QC2 AND QC3 AND QC4



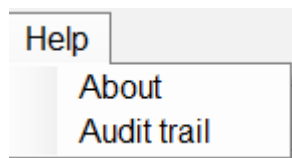
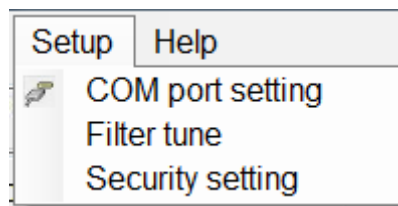
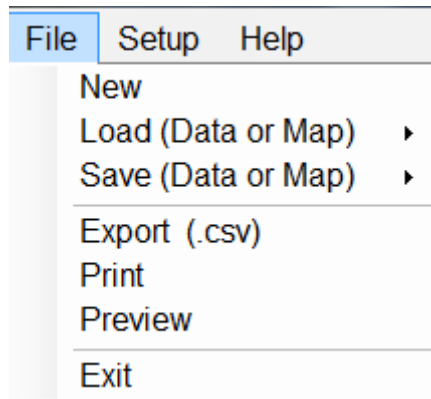
## Print options

Users can define the Project title, User name, experiment Note, and check the desired items in Sections for printing to print the result of the experiment.



## Main Menu Configuration

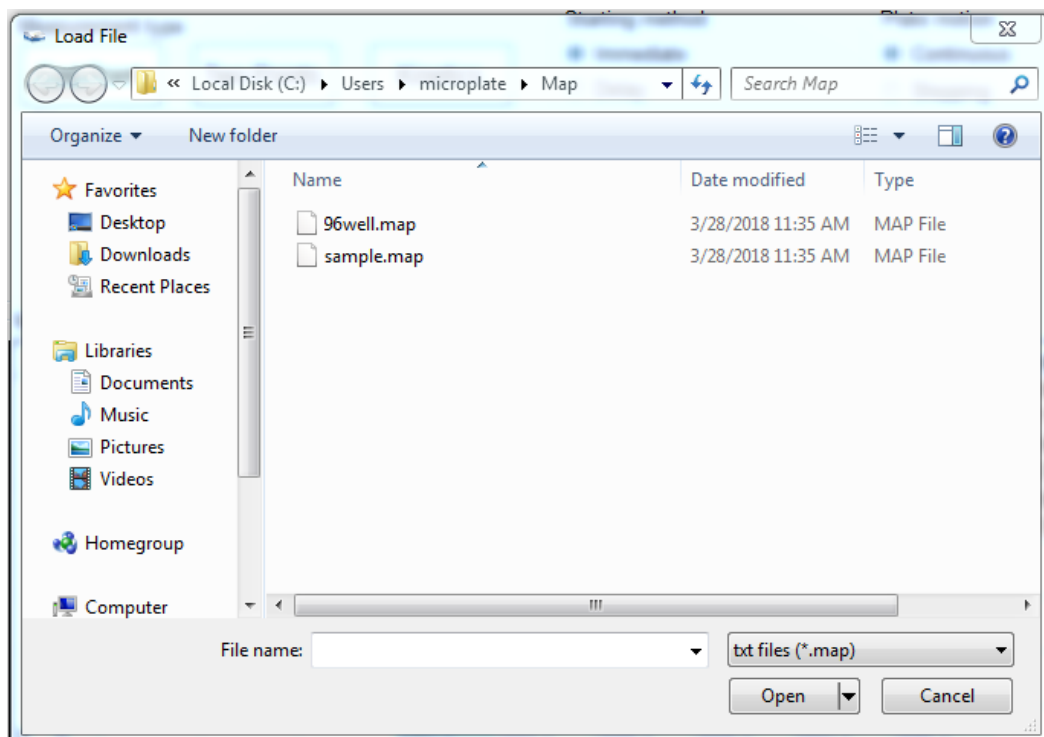
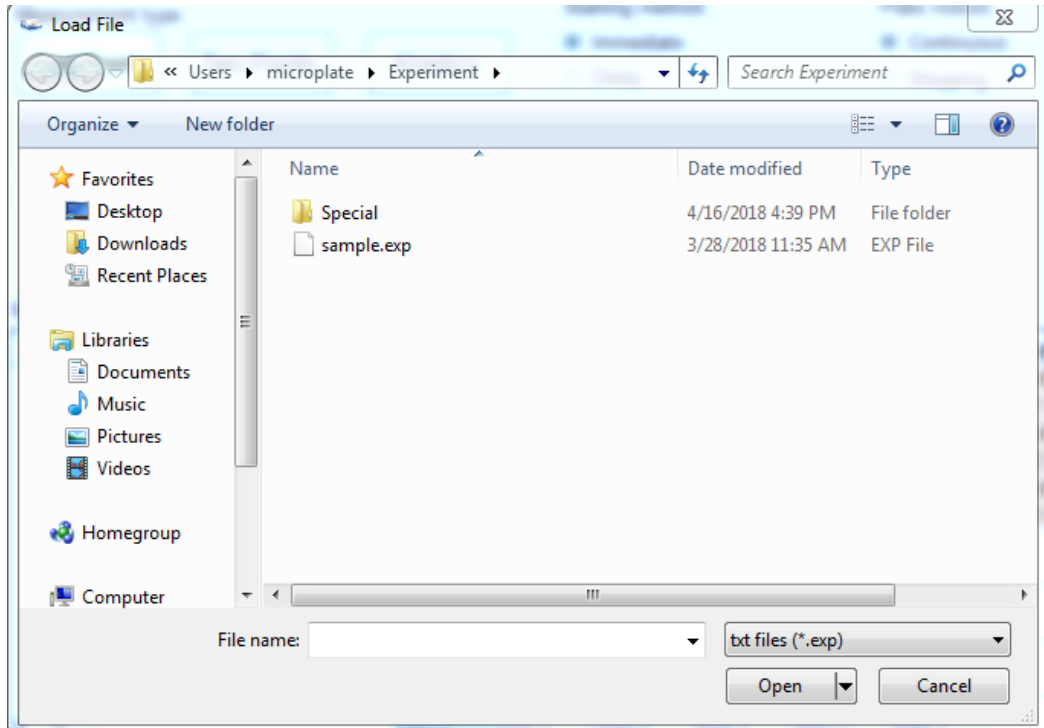
There are three functions on the main menu. They are File, Setup and Help.



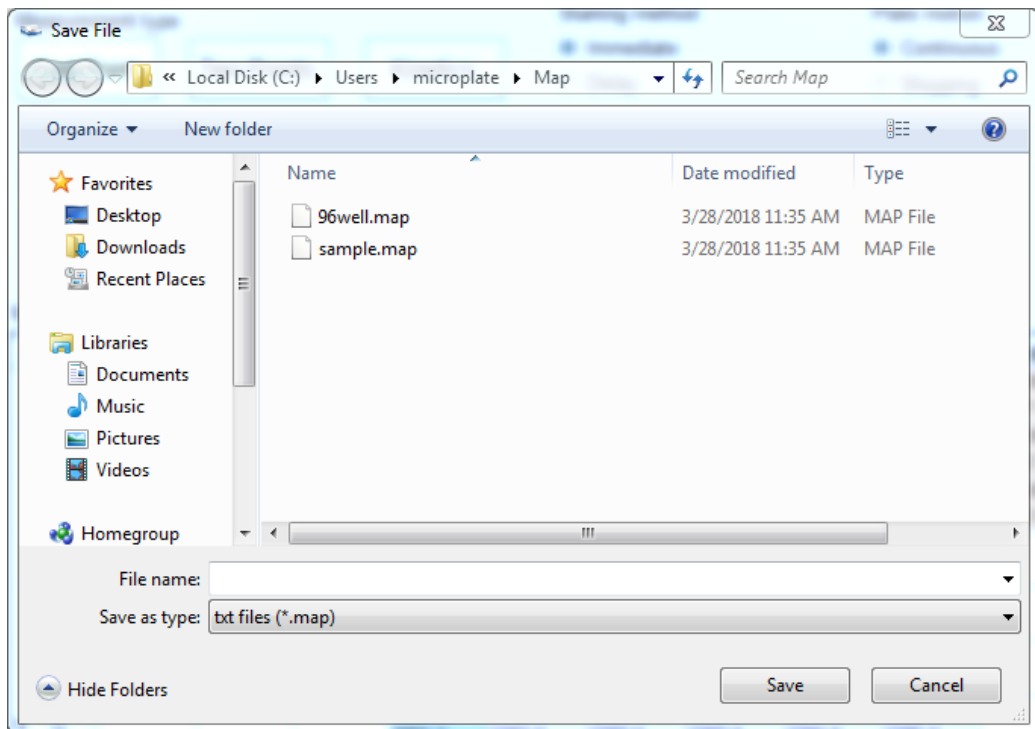
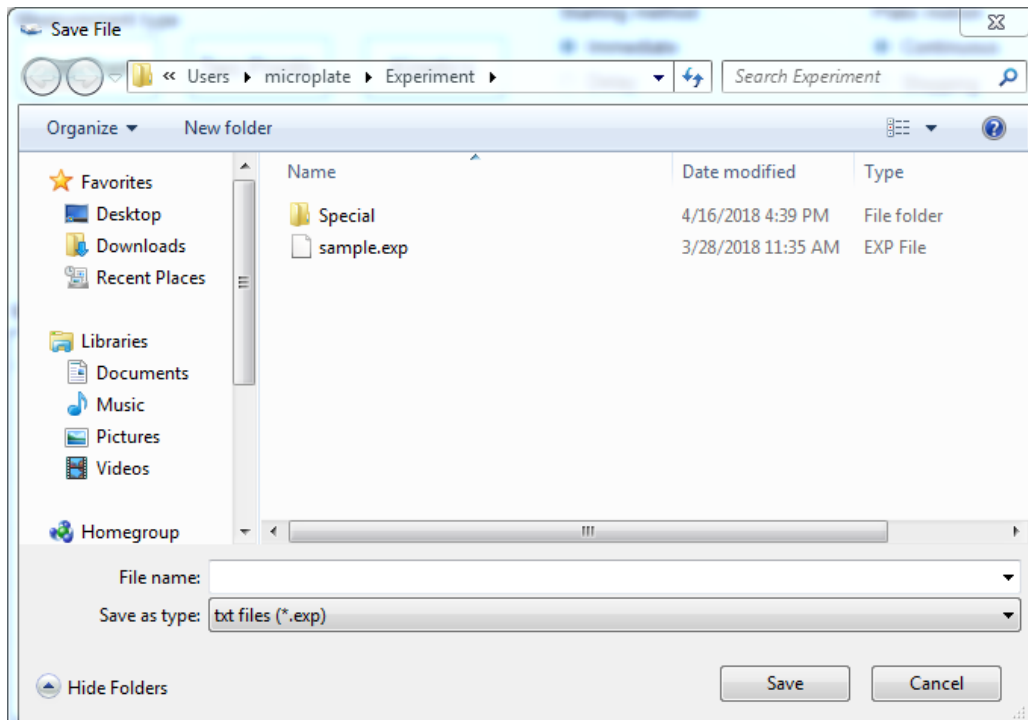
## File menu functions

There are seven options, i.e. New, Load (Data or Map), Save (Data or Map), Export (.csv), Print, Preview, and Exit under the main menu.

1. New: Create a new experiment
2. Load (Data or Map) : Load an existing experiment file or map layout



### 3. Save (Data or Map) : Save experiment file or map layout



4. Export (.csv) : To export to a file with file extension "csv", and it can be loaded into the excel, notepad or google spreadsheet
5. Print: To print report using the printer connected to the PC
6. Preview: To preview the experiment report before printing

Date of printing : 5/23/2018 3:49:30 PM Page : 1

Name :  
 User : admin  
 Note :

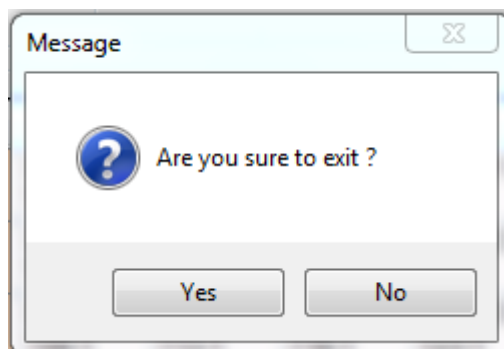
**Results data**

Protocol parameters  
 Experiment file: C:\Users\965Mate\Experiments\sample.exp  
 Measurement type: End point  
 Main\_1 filter(nm): 405  
 Starting method: Immediate  
 Plate mode: Continuous  
 Need shake: NO  
 Need incubator: NO  
 Need extrapolation: NO  
 Quant. method: Curve on plate  
 Quant. standards number: 5  
 Curve fit method: Linear regression

Plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	SAM01-1	SAM02-1	SAM03-1	SAM04-1	SAM05-1	SAM06-1	SAM07-1	SAM08-1	SAM09-1	SAM10-1	SAM11-1	SAM12-1
B	SAM01-2	SAM02-2	SAM03-2	SAM04-2	SAM05-2	SAM06-2	SAM07-2	SAM08-2	SAM09-2	SAM10-2	SAM11-2	SAM12-2
C	SAM01-3	SAM02-3	SAM03-3	SAM04-3	SAM05-3	SAM06-3	SAM07-3	SAM08-3	SAM09-3	SAM10-3	SAM11-3	SAM12-3
D				POS01-1	STD01-1	STD02-1	STD03-1	STD04-1	STD05-1			
E				POS01-2	STD01-2	STD02-2	STD03-2	STD04-2	STD05-2			
F				BLK01-1	STD01-3	STD02-3	STD03-3	STD04-3	STD05-3			
G				NEG01-1	STD01-4	STD02-4	STD03-4	STD04-4	STD05-4			
H				NEG01-2	STD01-5	STD02-5	STD03-5	STD04-5	STD05-5			
Raw abs.												
P1(Main)												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.000	0.000	0.000	0.232	0.397	0.486	1.199	2.321	2.860	0.000	0.000	0.259

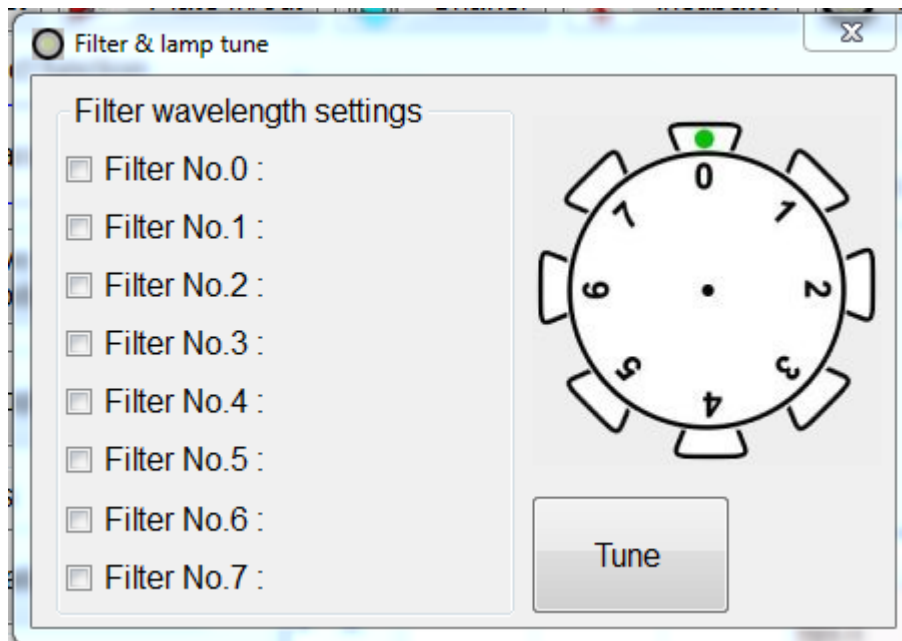
7. Exit: to end the AgileCon\_2.0 operation



## Setup menu functions

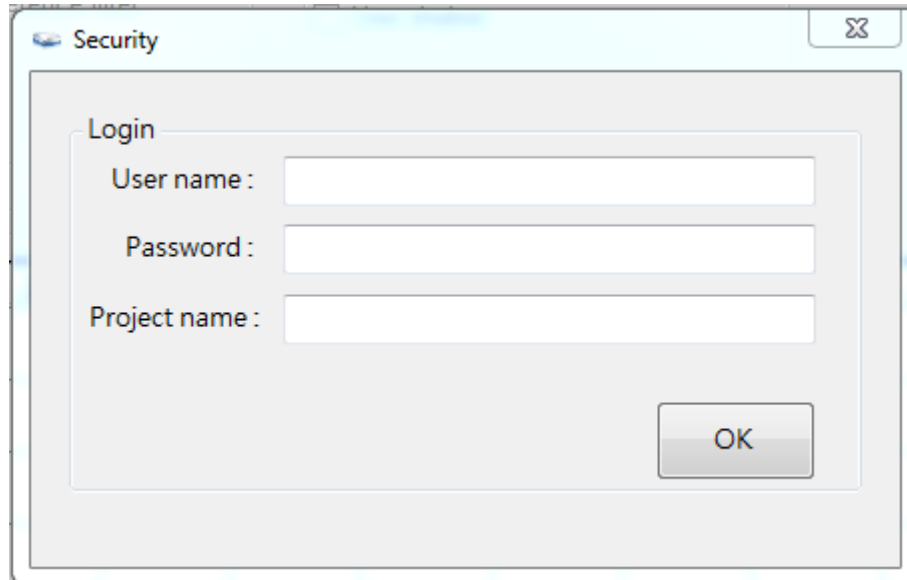
The setup menu includes COM port setting, filter tune, and the Security setting.

1. COM port setting: User can change the desired COM port to communicate between instrument and AgileCon\_2.0. The AgileCon\_2.0 can automatically detect all available COM ports on the PC.
2. Filter tune: The instrument has an eight- slot filter wheel for user to install filters. After installing new filters on the instrument, it is important to set the correct filter wavelength on the AgileCon\_2.0. Check the check box on the left to enter desired wavelength for the



filter, and press the Tune button after the desired wavelengths are entered.

3. Security setting: Users should log in with their own ID and Password to start the experiment. This function lets you log in / log out system, create, and or delete user ID.

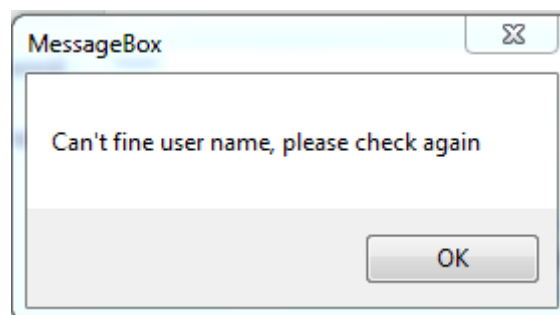


To enter the system, please use the default value (admin) to log in.

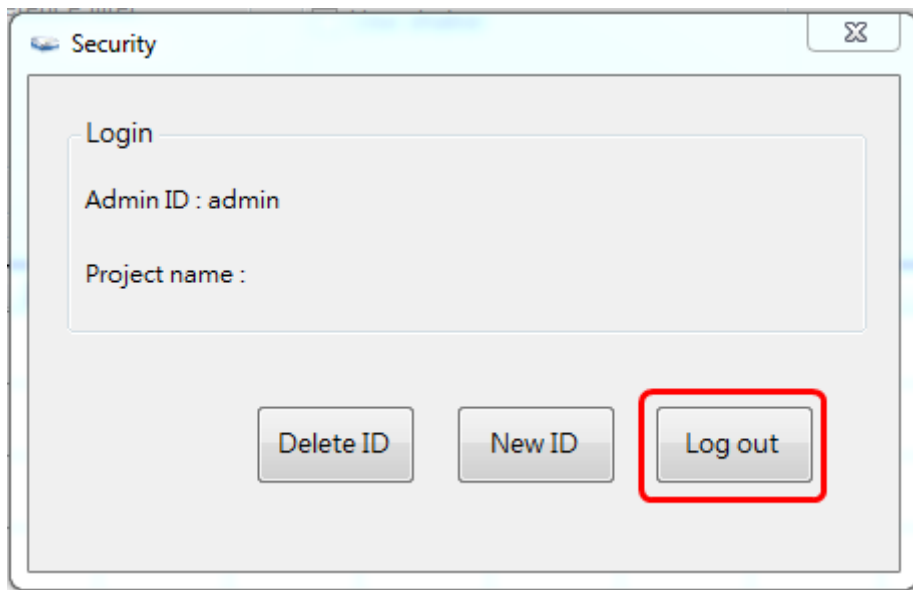
User name: admin

Password: admin

Entering the wrong user name or password, The AgileCon\_2.0 will show the pop-up window as below.

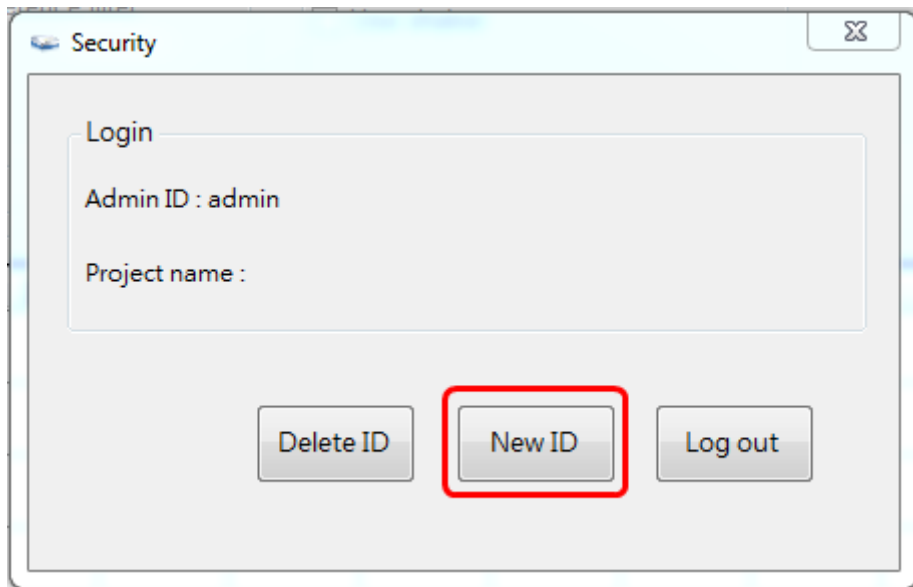


## Log out system



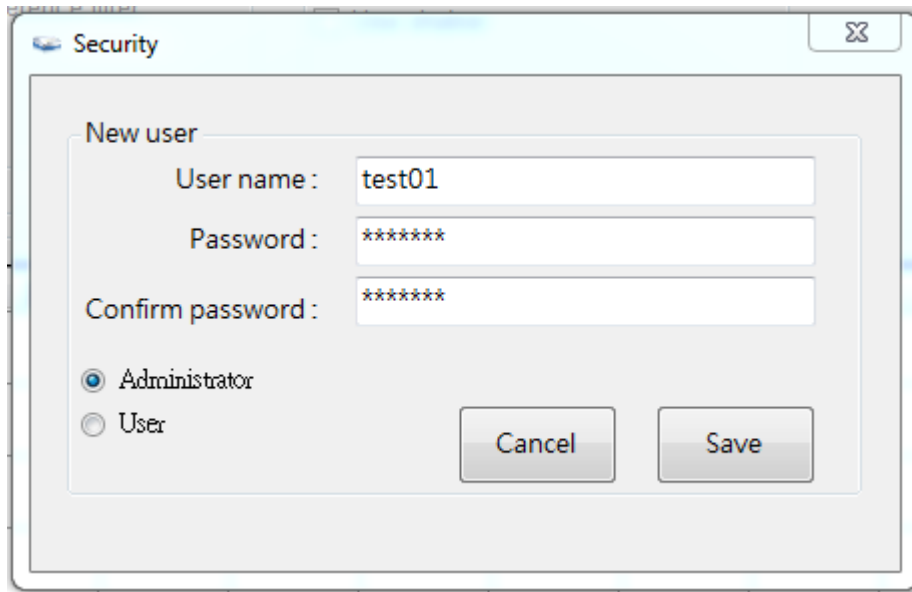
## Create a new ID

This function can be performed by users with administrator authority only.





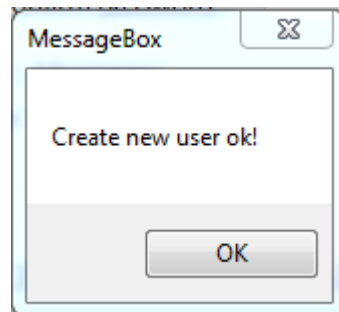
Enter the new ID with user name, password, and ID authority (administrator or user), and then press Save button to accept.



The image shows a 'Security' dialog box with a title bar containing a close button. The dialog is titled 'New user' and contains the following fields and controls:

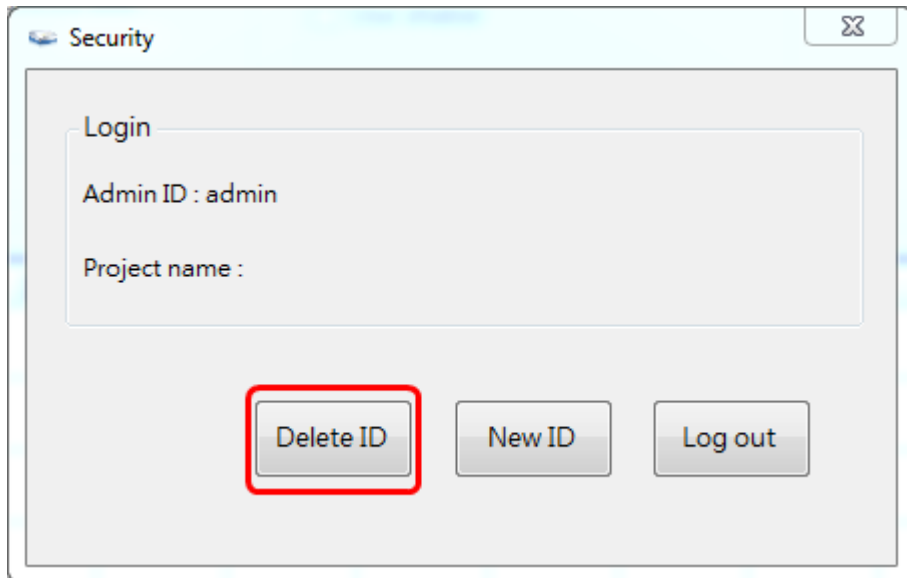
- 'User name : test01' (text input)
- 'Password : \*\*\*\*\*' (password input)
- 'Confirm password : \*\*\*\*\*' (password input)
- Radio buttons for 'Administrator' (selected) and 'User'.
- 'Cancel' and 'Save' buttons.

Successfully creating a new user will see the pop-up window as below.

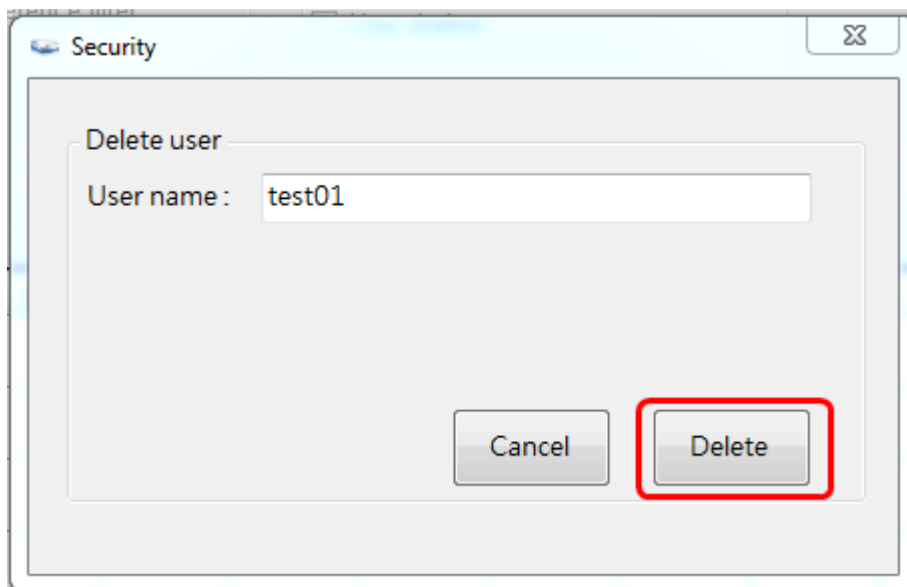


## Delete ID

This function can be performed by users with administrator authority only.



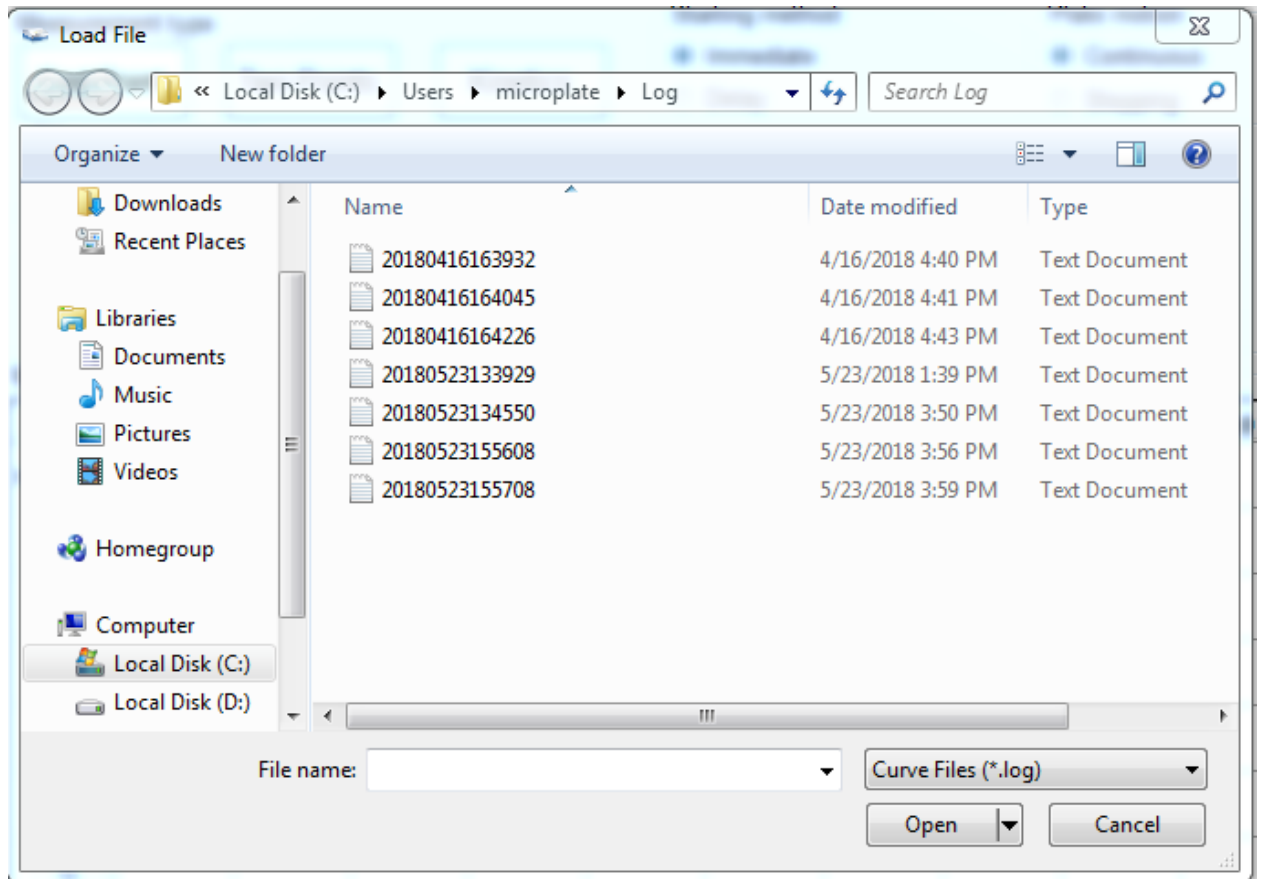
Enter the ID which you want to delete and press Delete to execute.



## Help menu functions

The help menu is trails the log-in and operation record.

1. Audit trail: To review the records of activities of different ID



## Toolbar Menu Configuration

There are Connect, Plate In/Out, Shaker, Incubator, Filter tune, and Post processing tabs on the toolbar menu.

1. Connect: To setup the COM port connection between the instrument and the PC.



2. Plate in/out: To move the plate holder in or out, the plate holder status will show on the status bar



3. Shaker: This tab is used to configure and operate the shaker. The shaker has three translation speeds, i.e. low (8Hz), Medium(11Hz) and High (14Hz)



4. Incubator: To warm up the incubator at set-point temperature from the lowest 15°C to the highest 50°C. If the ambient temperature is higher than 15°C, the effective lowest temperature should be set to the ambient temperature + 3C.



5. Filter tune: The AgileCon has an eight- slot filter wheel for user to install filters



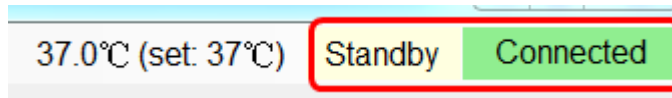
6. Post Processing: Use the current parameters of selected measure mode and recalculate the data



## Message Area Configuration

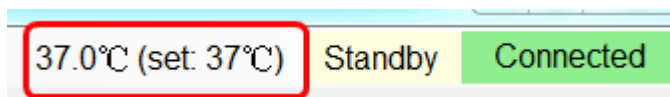
The message area contains two parts, the status message and the temperature monitor.

1. Status message: To display the status of instrument current operation condition. All messages are listed in the following chart.



Message	Description
Initializing	Initializes the instrument
Standby	Test ready
Data reading	Load data
Post processing	Recalculate the data
Disconnect	The instrument has no connection with AgileCon_2.0
Connected	The instrument connects with AgileCon_2.0
Filter tune	Start tuning filter

2. Temperature monitor: To display the real-time temperature and set-point temperature of instrument incubator.

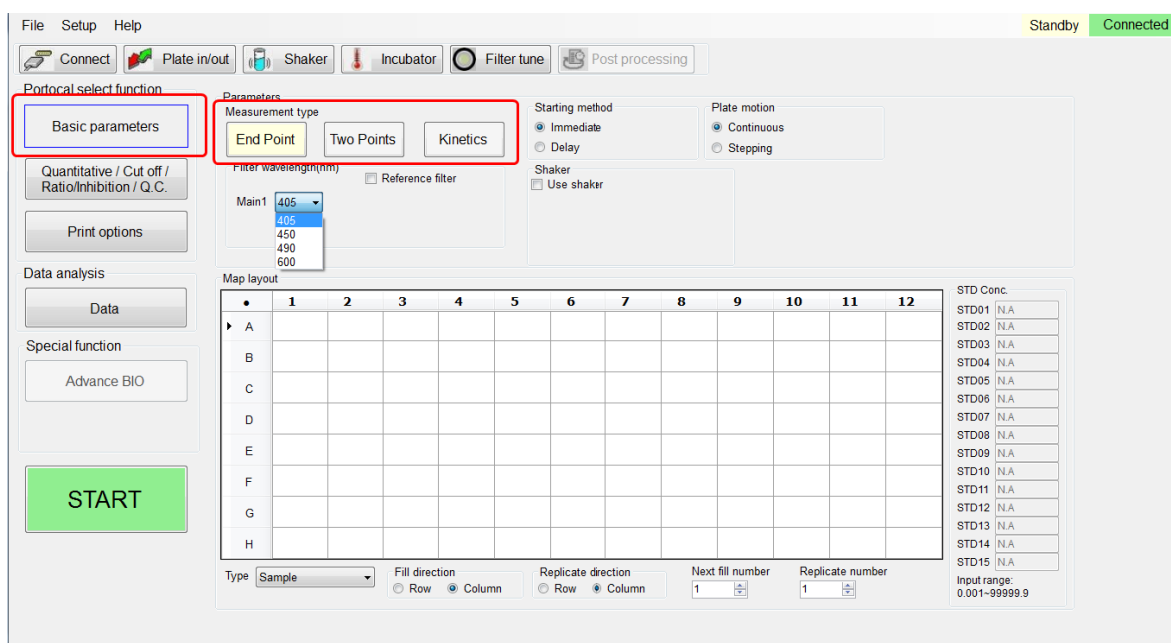


## Defining Parameters for Experiment

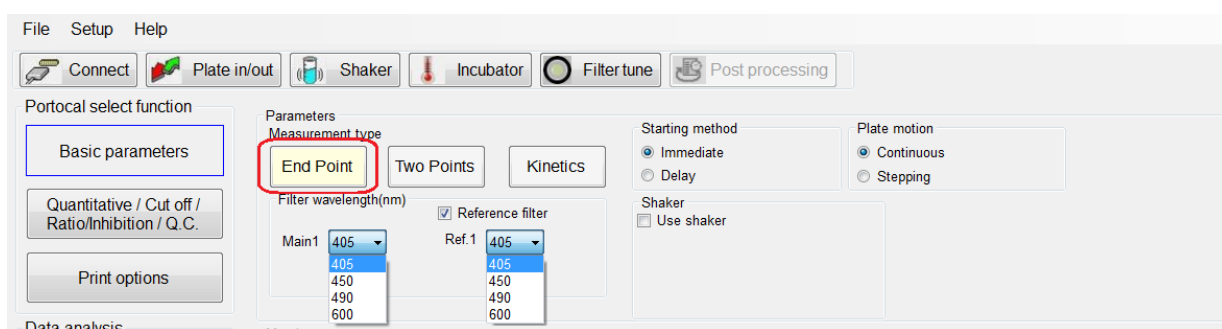
### Defining Parameters

When starting an experiment, users must first define the parameters such as wavelength, reading method, plate motion, incubator, and shaking. Above functions are included in Basic parameters tab.

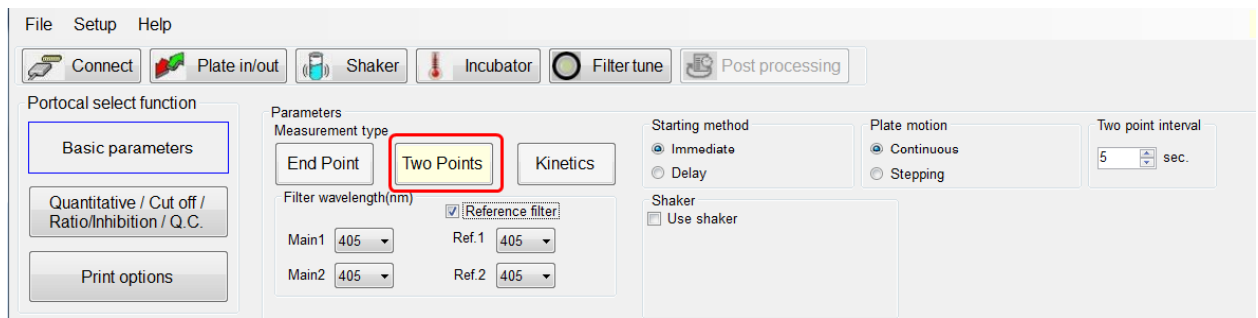
1. Measurement Type: Users can define three measuring types, i.e. End point, Two point and Kinetic.



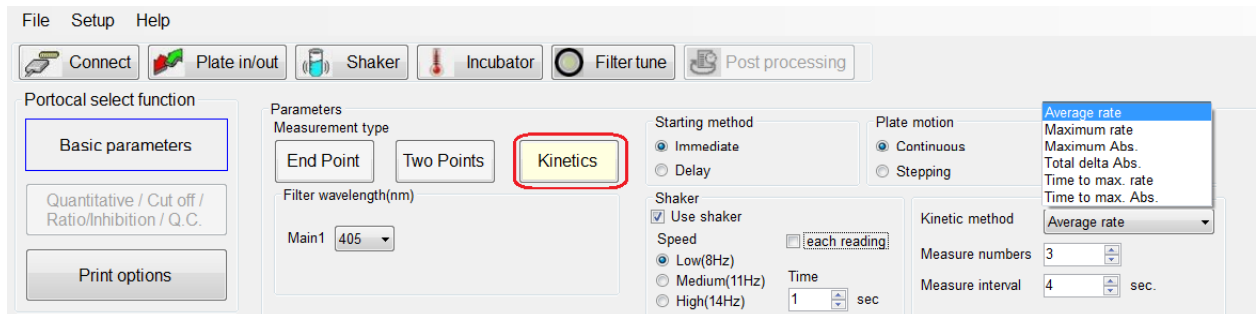
### a \ End Point



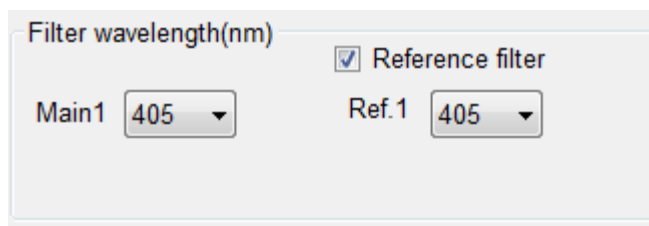
## b 、 Two Points



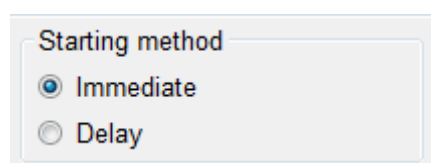
## c 、 Kinetics: Kinetics measuring method can only select main filter without reference filter



2. Filter wavelength: Users need to select the main filter wavelength for the desired experiment. In addition, users can also select a reference wavelength.

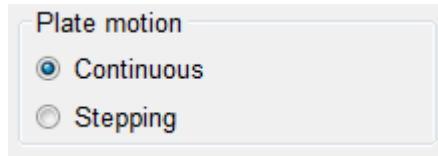


3. Starting method: Define when to start the selected experiment.
  - a 、 Immediate: Start measurement right after pressing the START tab
  - b 、 Delay: Users can define 0~999s as delay time before starting measurement.





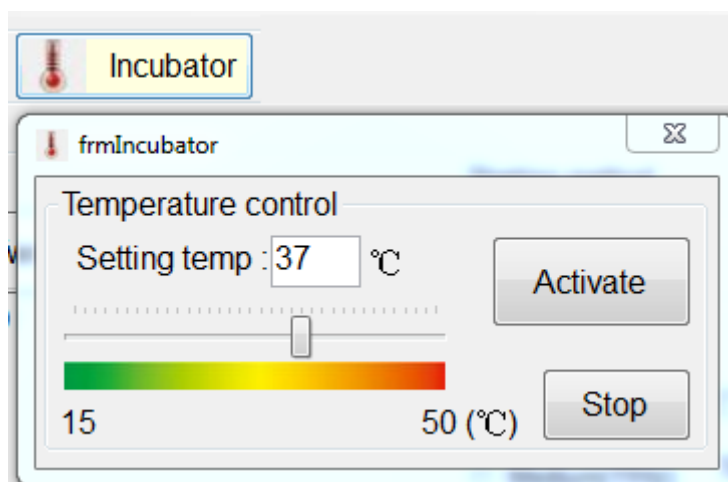
4. Plate motion: To define how the plate is moved when measuring
- a · Continuous: When measuring, the plate is translated smoothly during the entire motion stroke.



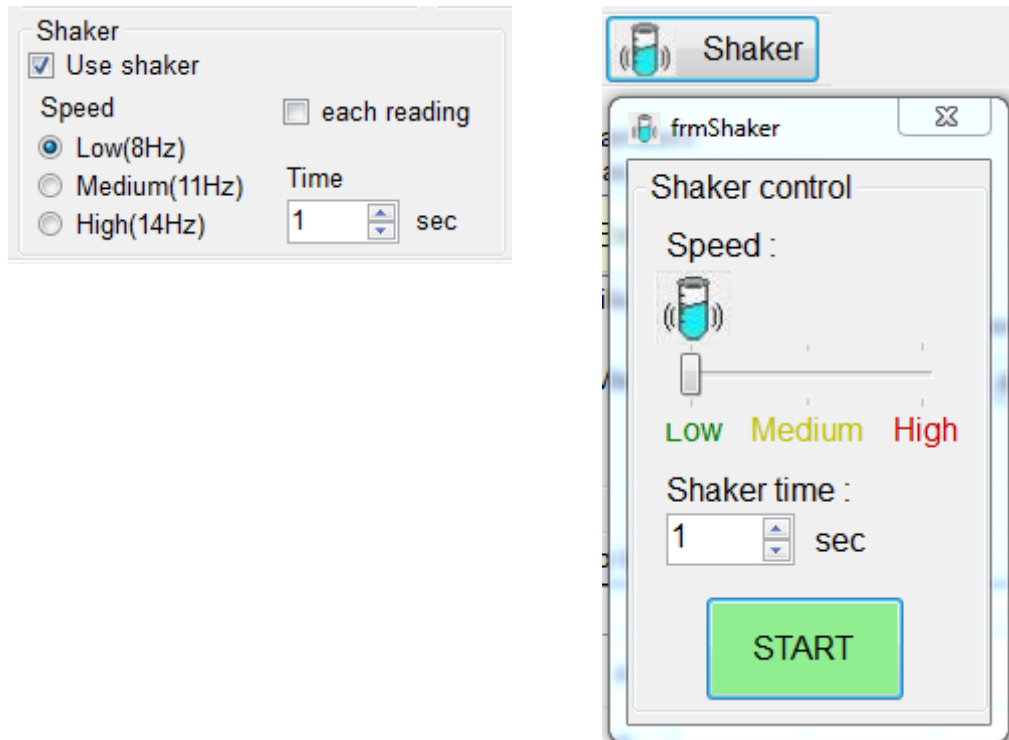
- b · Stepping: User can define the stepping intervals among 0~999 msec. In kinetic mode, there are variable stepping interval and fixed stepping interval to be selected.



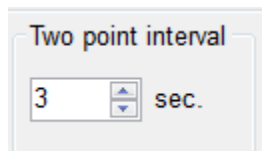
5. Incubation: Users can define the incubator temperature by clicking the incubation tab. The temperature can be set from ambient 15°C to 50°C.



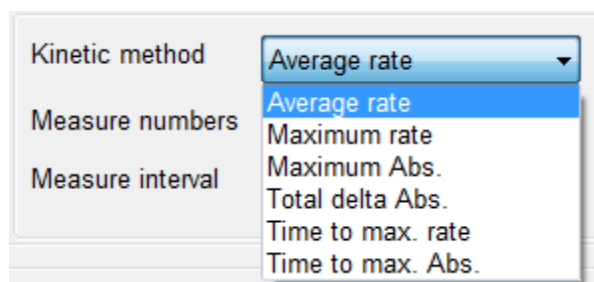
6. Shaker: The shaker of the instrument shakes with three types of speed, and the shaking time can be arranged among 0~999s



7. Two point interval: Users can select the two point interval among 3~999s



8. Kinetic method, numbers, and interval: In kinetic measurement mode, user can select the data calculation method, test cycles, and cycle interval.
- a. Kinetic method: Users can select Average rate, Maximum rate, Maximum OD, Total delta OD, Time to max slope, Time to max OD for mapped wells calculation.



- b 、 Measure number: User can enter the measuring numbers among 3~255 cycles.
- c 、 Measure interval: User can enter the measure interval. They are among 4~65535s in continuous motion, and 6~65535s in stepping motion.

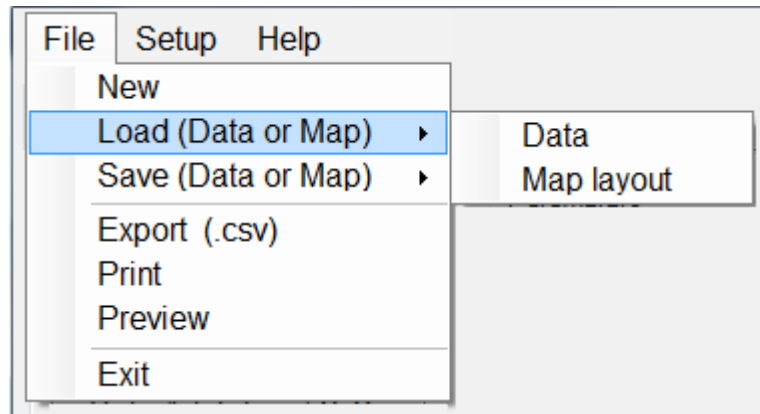
Kinetic method	<input type="text" value="Average rate"/>
Measure numbers	<input type="text" value="3"/>
Measure interval	<input type="text" value="4"/> sec.

## Well Mapping

The AgileCon\_2.0 provides five types of well for the user to define 96-well map. Moreover, the user can save the mapped wells and reload them for further uses.

### 1. Save and load map layout:

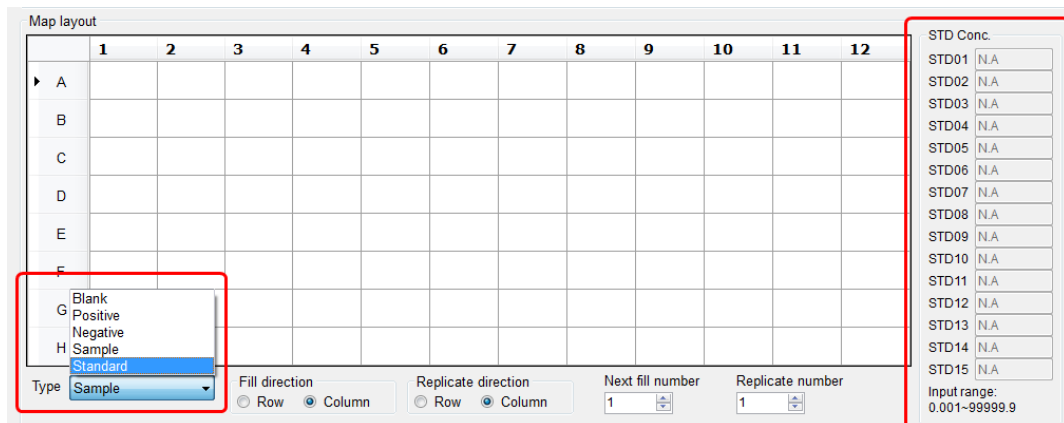
Users can load or save their map layout from File/ Load (Data or Map) or Save (Data or Map) functions



### 2. Well mapping method:

- a - Select the well type to be defined (Blank, Positive, Negative, Sample, Standard) on the map layout. On the right side, enter the concentration values if standard is selected.

Note: Sample is the only available type in kinetic mode.



- b. Select the fill and replicate directions, enter next fill number, and replicate number.

Fill direction

Row  Column

Replicate direction

Row  Column

- c. Use mouse to draw an area, which wells are to be placed with selected type.
- d. Right click on the mouse to select the fill option.

Map layout

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Fill

Modify

Clear

Clear Group

Clear All

Type: Standard

Fill direction:  Row  Column

Replicate direction:  Row  Column

Next fill number: 1

Replicate number: 1

STD Conc.

STD01	N.A
STD02	N.A
STD03	N.A
STD04	N.A
STD05	N.A
STD06	N.A
STD07	N.A
STD08	N.A
STD09	N.A
STD10	N.A
STD11	N.A
STD12	N.A
STD13	N.A
STD14	N.A
STD15	N.A

Input range: 0.001-99999.9

- e. The selected ten standards are thus located on the well map.

Map layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD01 C01-1	STD06 C06-1										
B	STD02 C02-1	STD07 C07-1										
C	STD03 C03-1	STD08 C08-1										
D	STD04 C04-1	STD09 C09-1										
E	STD05 C05-1	STD10 C10-1										
F												
G												
H												

Type: Standard

Fill direction:  Row  Column

Replicate direction:  Row  Column

Next fill number: 11

Replicate number: 1

STD Conc.

STD01	1.000
STD02	1.000
STD03	1.000
STD04	1.000
STD05	1.000
STD06	1.000
STD07	1.000
STD08	1.000
STD09	1.000
STD10	1.000
STD11	N.A
STD12	N.A
STD13	N.A
STD14	N.A
STD15	N.A

Input range: 0.001-99999.9

3. Fill and replicate well:
  - a. Fill direction: To number the sequence of selected sample type in column or row direction.
  - b. Replicate direction: To number the sequence of the replicates of selected sample type in column or row direction.

**Example of filling and replicating the well map**

	1	2	3	4	5	6	7
A		Start location	Sample x 2				
B			1-1	2-1			Replicate x 4
C			1-2	2-2			
D	Replicate (Column)		1-3	2-3			
E			1-4	2-4			
F							
G							

Fill (Row)

	1	2	3	4	5	6	7
A		Start location					
B			3-1	3-2	3-3	3-4	
C			4-1	4-2	4-3	4-4	Fill (Column)
D	Replicate x 4						
E							
F							
G							

Replicate (Row)

4. Blank, positive control, and negative control each has only one name (BLK01, POS01, NEG01).
5. Standard can be configured as 1~15 names(STD01~STD15)
6. Sample has 96 names most (SAM01~SAM96).

7. Types of well:

- a 、 BLK: Which is painted with light green background on the well map
- b 、 POS: Which is painted with light red background on the well map
- c 、 NEG: Which is painted with light blue background on the well map
- d 、 Sample: Which is painted with light orange background on the well map
- e 、 Standard: Which is painted with light purple background on the well map

Users must fill in the concentration values of selected standards in ascending or descending order.

Map layout												STD Conc.		
	1	2	3	4	5	6	7	8	9	10	11	12		
A	BLK01 Z01-1	BLK01 Z01-2	BLK01 Z01-3	BLK01 Z01-4	BLK01 Z01-5								STD01	1.000
B	POS01 P01-1	POS01 P01-2	POS01 P01-3	POS01 P01-4	POS01 P01-5								STD02	1.000
C	NEG01 N01-1	NEG01 N01-2	NEG01 N01-3	NEG01 N01-4	NEG01 N01-5								STD03	1.000
D	SAM01 T01-1	SAM02 T02-1	SAM03 T03-1	SAM04 T04-1	SAM05 T05-1								STD04	1.000
E	STD01 C01-1	STD02 C02-1	STD03 C03-1	STD04 C04-1	STD05 C05-1								STD05	1.000
F													STD06	N.A
G													STD07	N.A
H													STD08	N.A

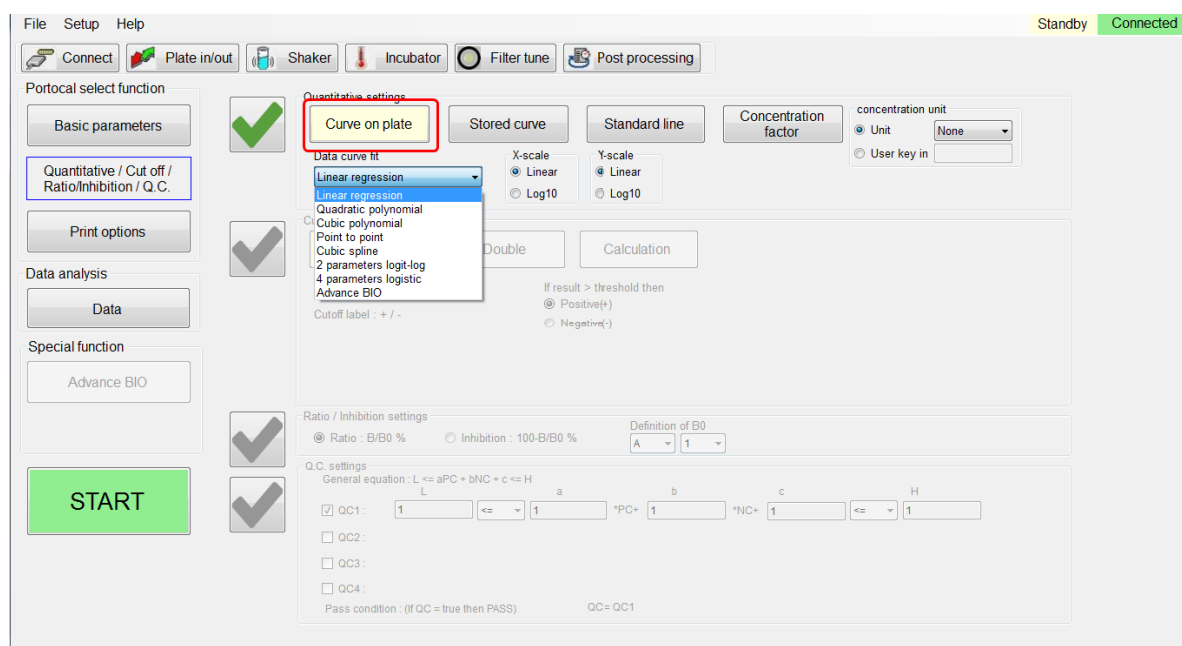
  

Type	Standard	Fill direction	<input type="radio"/> Row <input checked="" type="radio"/> Column	Replicate direction	<input type="radio"/> Row <input checked="" type="radio"/> Column	Next fill number	6	Replicate number	1	Input range:	0.001-99999.9
------	----------	----------------	---	---------------------	---	------------------	---	------------------	---	--------------	---------------

## Quantitative Measuring Method

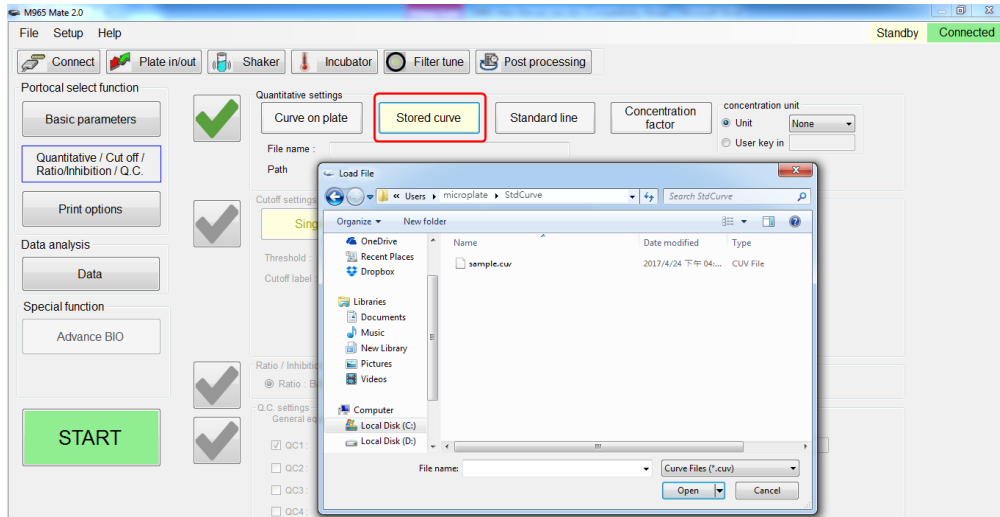
The AgileCon\_2.0 provides four types of Quantitative method, i.e. Curve on plate, Stored curve, Standard line, and Concentration factor.

1. Curve on plate: Use the standard on the well plate for the calibration curve calculation. There are seven types of curve fitting equations on the AgileCon\_2.0
  - a. Linear regression
  - b. Quadratic polynomial
  - c. Cubic polynomial
  - d. Point to point
  - e. Cubic spline
  - f. 2 parameters logit-log
  - g. 4 parameters logistic
  - h. Advance BIO (Option function)





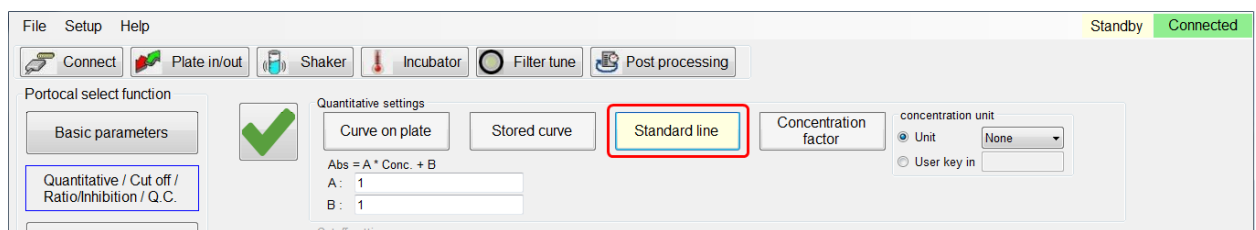
2. Stored curve: Users can load their stored curve for quantitative measurement, these curves with file extension ".cuv" are stored under directory AgileCon 2.0\StdCurve.



3. Standard line: User can use the  $Abs=A * Conc+B$  equation, and enter the values of A and B to calculate a standard line.

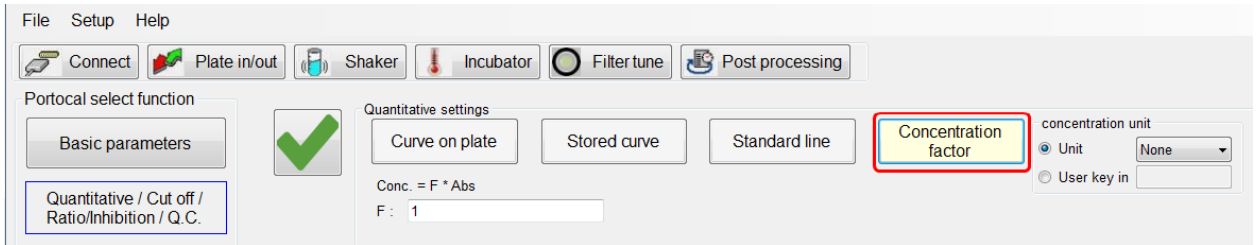
The value of A can be : -999999.999 ~ +999999.999

The value of B can be : -999999.999 ~ +999999.999

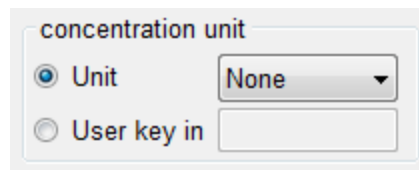


4. Concentration factor: User can enter a factor for calculating the concentration.

The value of F can be : -999999.999 ~ +999999.999



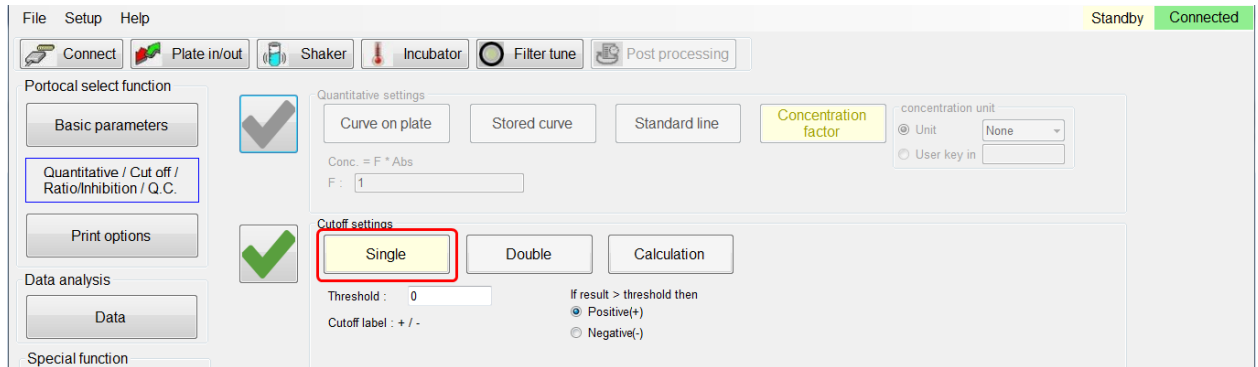
5. Measurement unit: Users can select 15 types of measurement unit "None", "G/dL", "U/L", "G/L", "ug/dL", "ABS", "mg/dL", "OD", "mABS", "U/mL", "ug/mL", "mEq/L", "mmol/L", "umol/L", "ng/mL". When "None" is selected, user can enter the desired measurement unit



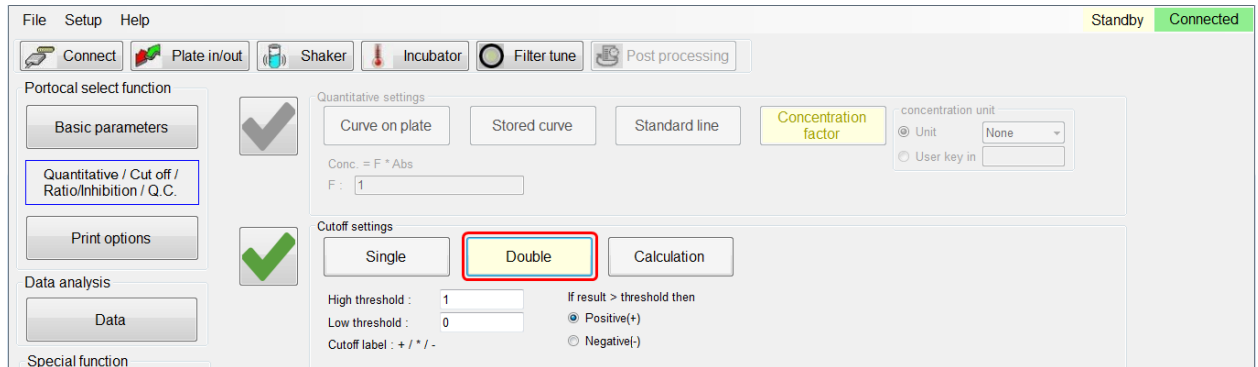
## Cutoff Measuring Method

The AgileCon\_2.0 provides three types of Cutoff measuring method.

1. Single cutoff method: User can enter a threshold of 0.0000~4.0000, and define OD result to be positive or negative.



2. Double cutoff method: Users can define the high and low thresholds. The high and low values can be among 0.0000~4.0000. The AgileCon\_2.0 determines OD results that are higher than high threshold, lower than low threshold, or between low and high thresholds to be positive(+), negative(-), or in-between (\*) respectively.



3. Calculation cutoff method: User can create a maximum of four formulas as the thresholds calculation and categorize the OD readings into 5 groups.

The equation listed below is applied to construct the thresholds with given a, b and c values.

$$EQ_n = a * PC + b * NC + c,$$

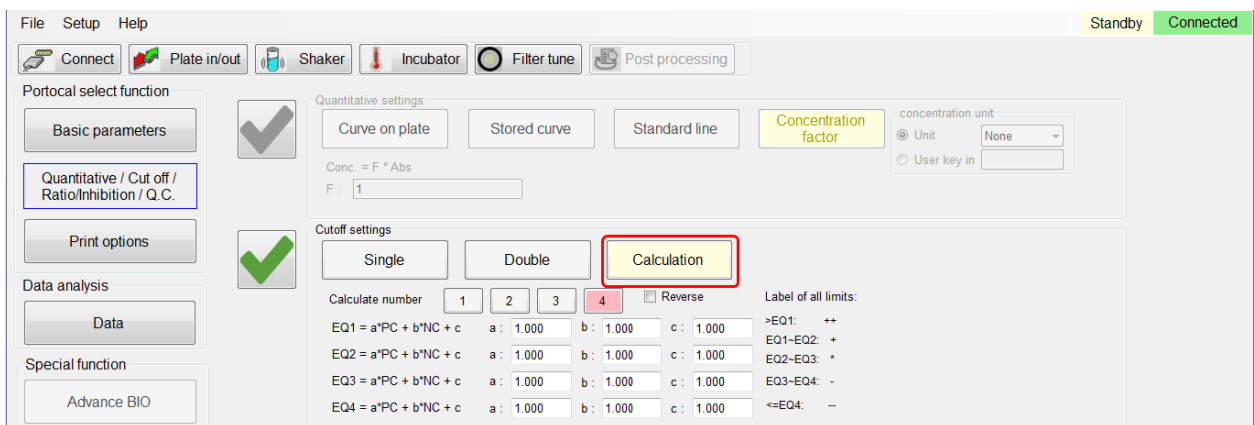
where **PC** means Positive Control, and **NC** means Negative Control.

The value of a, b and c can be -1000.000 ~ +1000.000

The calculated threshold values must follow the rule below:

$$EQ1 > EQ2 > EQ3 > EQ4$$

Example: With four thresholds applied, the OD reading higher than EQ1, between EQ1 and EQ2, between EQ2 and EQ3, between EQ3 and EQ4, or below EQ 4 is labeled by "++", "+", "\*", "-", or "--" respectively.



## Ratio/Inhibition Calculation Method

Select B0 as the standard value to calculate the rest of the plate well Bn

### 1. Ratio/Inhibition operating procedure

- a、 Ratio =  $(B_n/B_0)\%$
- b、 Inhibition =  $100\% - (B_n/B_0)\%$
- c、 Must have sample on B0 position or the AgileCon\_2.0 will show error
- d、 If the selected B0 has replicate number greater than one, the actual B0 value will be the average reading of this sample.
- e、 If B0 value is 0, the AgileCon\_2.0 will show error
- f、 If ratio is over 200%, the AgileCon\_2.0 will show HI; if lower than -200%, the AgileCon\_2.0 will show LO

The screenshot displays the AgileCon\_2.0 software interface. The top menu bar includes 'File', 'Setup', and 'Help'. On the right, the status is 'Standby' and 'Connected'. The main interface is divided into several sections:

- Portocal select function:** Includes 'Basic parameters', 'Quantitative / Cut off / Ratio/Inhibition / Q.C.' (highlighted with a blue border), 'Print options', 'Data analysis', and 'Special function'.
- Quantitative settings:** Features buttons for 'Curve on plate', 'Stored curve', 'Standard line', and 'Concentration factor'. It includes a formula  $Conc. = F * Abs$  and a field for 'F' set to '1'. There are also options for 'concentration unit' (Unit or User key in) and a dropdown for 'Unit' set to 'None'.
- Cutoff settings:** Includes buttons for 'Single', 'Double', and 'Calculation'. It has a 'Threshold' field set to '0' and options for 'If result > threshold then' (Positive(+) or Negative(-)).
- Ratio / Inhibition settings:** This section is highlighted with a red box. It contains radio buttons for 'Ratio : B/B0 %' and 'Inhibition : 100-B/B0 %' (selected). It also has a 'Definition of B0' section with a dropdown set to 'A' and a field set to '1'.
- Q.C. settings:** Includes a 'General equation'  $L = aPC + bNC + c$  and a 'Pass condition' '(If QC = true then PASS)'. It features checkboxes for 'QC1', 'QC2', 'QC3', and 'QC4', with 'QC1' checked. The equation is set to  $1 <= 1 * PC + 1 * NC + 1 <= 1$ .

A large green 'START' button is located at the bottom left of the interface.

## Q.C. Calculation Method

The purpose of the QC Calculation Method is to determine the reliability of the experiment.

1. At most 4 equations are applied to obtain the calculation results, QC1, QC2, QC3 and QC4.
2. Combining above QCs with logic operators OR, AND, and XOR to obtain the QC calculation result. The truth or falseness of QC decides the experiment to be pass or fail.
3. The value of a can be -1000.000 ~ +1000.000
4. The value of b can be -1000.000 ~ +1000.000
5. The value of c can be 1000.000 ~ +1000.000
6. The value of H can be -9999999.999 ~ +9999999.999
7. The value of L can be -9999999.999 ~ +9999999.999

File Setup Help Standby Connected

Connect Plate in/out Shaker Incubator Filter tune Post processing

Portocal select function

Basic parameters

Quantitative / Cut off / Ratio/Inhibition / Q.C.

Print options

Data analysis

Data

Special function

Advance BIO

START

Quantitative settings

Curve on plate Stored curve Standard line Concentration factor

Conc. = F \* Abs

F: 1

concentration unit

Unit: None

User key in

Cutoff settings

Single Double Calculation

Threshold: 0

If result > threshold then

Positive(+)

Negative(-)

Ratio / Inhibition settings

Ratio: B/B0 % Inhibition: 100-B/B0 %

Definition of B0

A: 1

Q.C. settings

General equation:  $L \leq aPC + bNC + c \leq H$

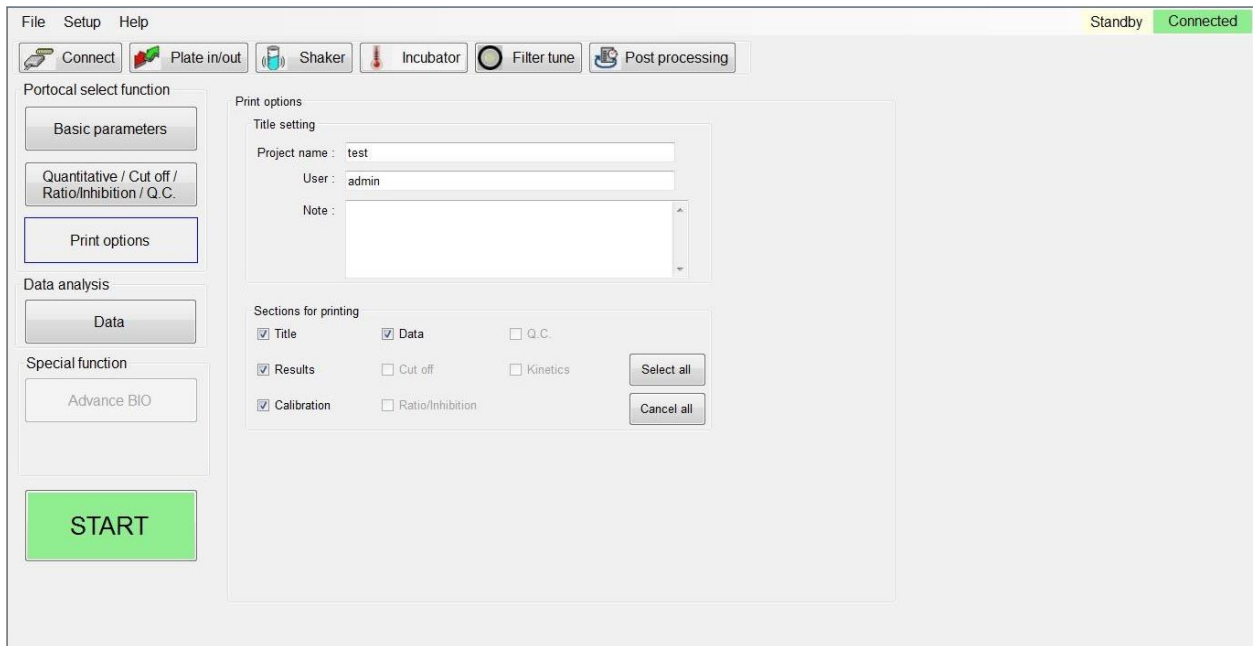
	L		a		b		c		H
<input checked="" type="checkbox"/> QC1	1	<=	1	*PC+	1	*NC+	1	<=	1
<input checked="" type="checkbox"/> QC2	1	<=	1	*PC+	1	*NC+	1	<=	1
<input checked="" type="checkbox"/> QC3	1	<=	1	*PC+	1	*NC+	1	AND	1
<input checked="" type="checkbox"/> QC4	1	<=	1	*PC+	1	*NC+	1	OR	1

Pass condition: (If QC = true then PASS)

QC = QC1 AND QC2 AND QC3 OR QC4

## Printing Options

Users can input project name, operator name, and experiment note to differentiate experiment reports. Users can also check boxes in the Section for printing to determine which items need be printed on the report.



## Interpreting the Results

The AgileCon\_2.0 will generate the result data after the experiment is completed. Press the Data tab on the left window, and select tab Results, Calibration, Data, Cutoff, Ratio/ Inhibition, Q.C, or Kinetic to view their experiment results.

1. Results: Click on the Results tab to review the parameter setup, plate layout, Raw OD, and Con Matrix of the experiment.

The screenshot shows the software interface with the 'Results' tab highlighted. The protocol parameters are as follows:

Protocol parameters	
Experiment file:	C:\Users\96
Measureme type:	End point
Main_1 filter(nm):	405
Starting method:	Immediate
Plate mode:	Continuous
Need shake:	NO
Need incubator:	NO
Need extrapolator:	NO
Quant. method:	Curve on plate
Quant. standards:	6
Curve fit method:	Linear regression

The plate layout table is shown below:

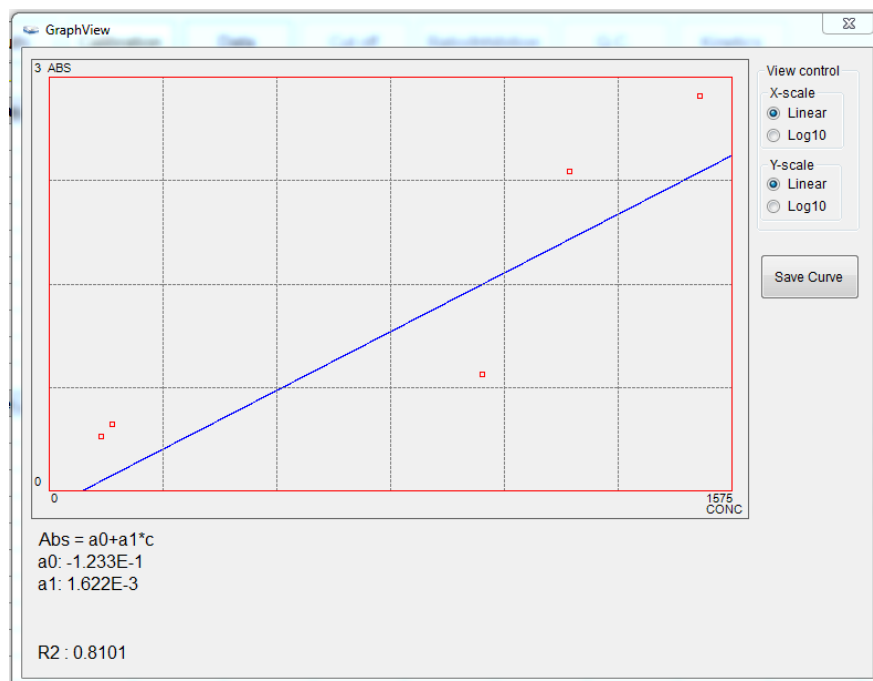
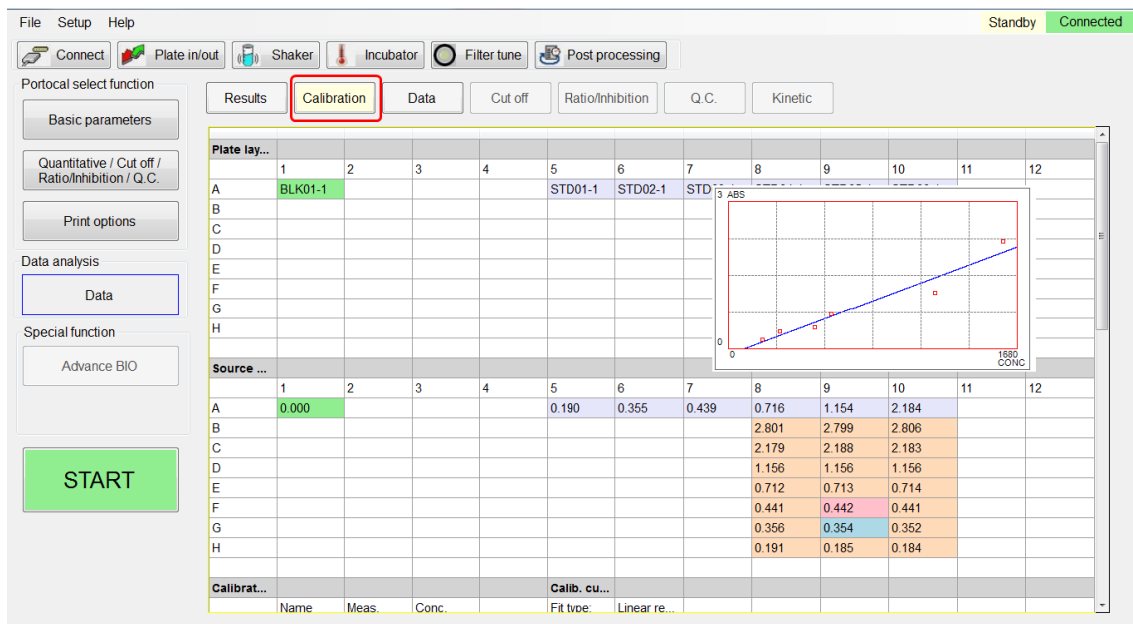
Plate layout	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK01-1					STD01-1	STD02-1	STD03-1	STD04-1	STD05-1	STD06-1	

2. Calibration: When Quantitative is checked, calibration curve will be displayed according to the setting parameters.
  - a. Layout: Shows the well mapping layout of the plate. Different types of well uses a different color to represent.
  - b. Source data: Shows the source data for the quantitative measurement.
    - i. In end point measurement, if there is no reference filter then the main filter (M1) data is the source data. If there is reference filter then M1 – R1 is the source data.
    - ii. In Two points measurement if there is no reference filter, the source data will be M1
    - iii. In Two points measurement if there is reference filter then the source data will be D1=M1-R1
    - iv. During Kinetic measurement, user cannot use reference filter, the M1 data will be the source data
  - c. Calibrators: Use C01~C15 to represent each STD's name and



OD value, and show the average measurement and the standard concentration value.

- d · Calib Curve: When using standard curve (Curve on plate or stored curve), apply selected fitting method to create a standard curve and its coefficients.
  - e · Residuals table: Use C01~C15 to show standard concentration values (C set), Average Abs, calculated concentration (Ccal), and their difference (Ccal-Cset).
  - f · Curve Viewer: User can double click on the curve to enable the curve viewer. User can also store the curve by pressing the Save Curve tab on the right.
- The default curve is stored in AgileCon\_2.0\StdCurve directory.



3. Data sheet: The raw data and calculated results of entire mapped wells can be listed in one data sheet. The sheet provides information about Name, Well ID, Replicate numbers, Abs, SD, CV%, Conc, Measuring unit, Cutoff, and Inhibition %. The average of replicated data is displayed by "\_avg" next to the well ID.

Name	well	Replicate	Abs.	SD	CV%	Conc.	Unit	Cutoff	R/I(%)
<b>POS.CONTR...</b>									
POS1	F9	1	0.442	---	---	---	---		
POS1_avg	---	---	0.442	0.000	ERR	429.127	G/dL	N/A	N/A
<b>NEG.CONTR...</b>									
NEG1	G9	1	0.354	---	---	---	---		
NEG1_avg	---	---	0.354	0.000	ERR	361.808	G/dL	N/A	N/A
<b>SAMPLES</b>									
SAM1	H8	1	0.191	---	---	---	---		
SAM1	H9	2	0.185	---	---	---	---		
SAM1	H10	3	0.184	---	---	---	---		
SAM1_avg	---	---	0.187	0.003	1.66	231.762	G/dL	N/A	N/A
SAM3	B8	1	2.801	---	---	---	---		
SAM3_avg	---	---	2.801	0.000	ERR	2233.713	G/dL	N/A	N/A
SAM4	C8	1	2.179	---	---	---	---		
SAM4_avg	---	---	2.179	0.000	ERR	1757.896	G/dL	N/A	N/A
SAM5	D8	1	1.156	---	---	---	---		
SAM5_avg	---	---	1.156	0.000	ERR	975.322	G/dL	N/A	N/A
SAM6	E8	1	0.712	---	---	---	---		
SAM6_avg	---	---	0.712	0.000	ERR	635.671	G/dL	N/A	N/A
SAM7	F8	1	0.441	---	---	---	---		
SAM7_avg	---	---	0.441	0.000	ERR	428.362	G/dL	N/A	N/A
SAM8	G8	1	0.356	---	---	---	---		
SAM8_avg	---	---	0.356	0.000	ERR	363.338	G/dL	N/A	N/A
SAM10	B9	1	2.799	---	---	---	---		

4. Cutoff results: Clicking the Cutoff tab, the AgileCon\_2.0 shows the cutoff symbols on mapped wells. Depending on the conditions, there will be five symbols to represent the cutoff results.

{ ++ } 、 { + } 、 { \* } 、 { - } 、 { -- }

	1	2	3	4	5	6	7	8	9	10	11	12
A	*				*	*	*	*	+	+		
B								+	+	+		
C								+	+	+		
D								+	+	+		
E								*	*	*		
F								*	*	*		
G								*	*	*		
H								*	*	*		

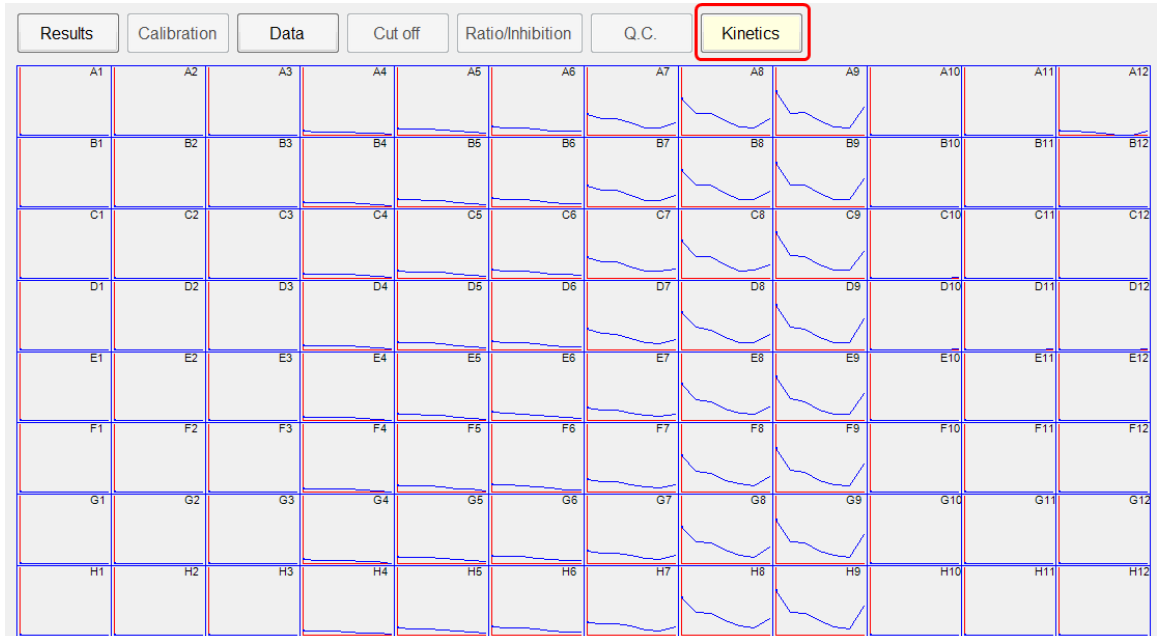
5. Ratio/Inhibition results: Clicking the Ratio/Inhibition tab, the AgileCon\_2.0 shows ratio or inhibition values of mapped wells. Data higher than 200% is shown Hi, and lower than -200% is shown LO.

	1	2	3	4	5	6	7	8	9	10	11	12
▶ A	100.00%			-809.09%	56.36%	19.09%	0.00%	-62.95%	-162.27%	-396.14%	-533.41%	
B								-534.55%	-534.32%	-536.36%		
C								-394.09%	-397.05%	-395.68%		
D								-163.18%	-162.73%	-162.73%		
E								-61.82%	-62.05%	-62.27%		
F								-0.23%	-0.45%	0.00%		
G								19.09%	19.55%	20.00%		
H								56.59%	57.95%	58.18%		

6. Q.C results: Clicking the QC calculation method, the AgileCon\_2.0 shows the QC criteria, Pass condition, and Result on the data sheet.

	1	2	3	4	5	6	7	8	9	10	11	12
<b>Quality controls</b>												
Controls:												
	Control	abs.	conc.									
	PC	0.442	---									
	NC	0.354	---									
Criteria:												
		L		a		b		c		H		
	QC1:	0.4	<=	1.000	*PC	0	*NC	0	<=	0.5		
	QC2:	0.3	<=	0	*PC	1.000	*NC	0	<=	0.4		
	QC3:	0.6	<=	1.000	*PC	1.000	*NC	1.000	<=	1		
Pass condition:												
	if QC = TRUE then PASS											
	QC =	QC1	AND	QC2	OR	QC3						
Result:												
	QC1:	PASS										
	QC2:	PASS										
	QC3:	FAIL										
	QC:	PASS										

7. Kinetic results: When using the kinetic measuring method, AgileCon\_2.0 will display the kinetic curves for each mapped wells. User can check the reaction rate easily on this screen.



Double click on the selected well to show a detailed view of the well number and OD value at selected sampling number.

