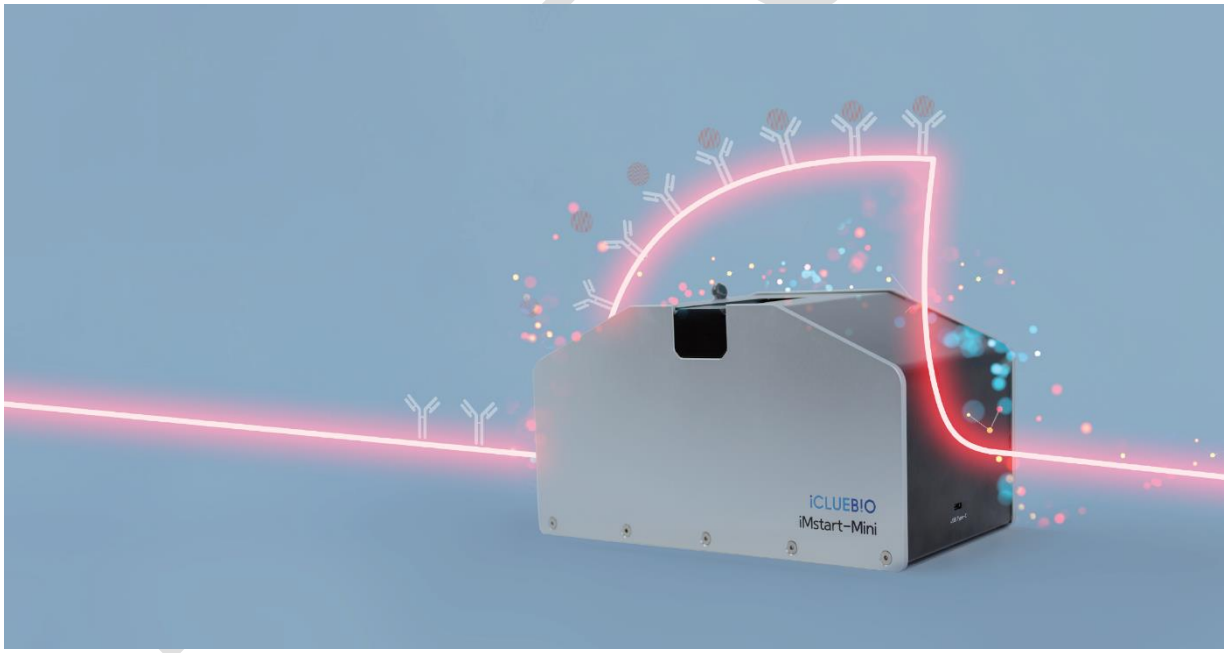
iMSPR-mini

Handbook

With basic training kit (amine coupling)

Guidebook for beginners



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Introduction

The purpose of this handbook is for researchers to learn how to operate SPR sensors and learn the basics for more complex applications by performing ligand immobilization and intermolecular binding analysis using iMSPR-Mini device, measurement software, and analysis software (Tarcedraw)

The experiments in this handbook were explained on the premise that the basic training reagent set provided by iCLUEBiO was used.

The basic training reagent set is designed to train intermolecular binding assays using SPR sensors.

Device and Materials

- iMSPR-mini device
- 2-channel tubing pump (peristaltic pump)
- 2-channel bubble removing device (degasser)
- PC for monitoring (for Microsoft Windows)
- Basic training kit
- Experiment assistant tools (matching oil, tweezer, pipet(tips), reagent tube)

Components	Details
COOH Au chip x 3	COOH surface sensor chip
Running Buffer (30 ml) x 1	PBS (10X), pH 7.4
Immobilization Buffer (30 ml) x 1	10 mM sodium acetate, pH 4.0
Activation solution 1 (200 ul) x 3	100 mM NHS
Activation solution 2 (200 ul) x 3	200 mM EDC
Ligand (50 ul) x 1	Recombinant protein A
Analyte (1 ml) x 1	Purified human IgG
Blocking solution (1 ml) x 1	1 M ethanolamine
Regeneration solution (1 ml) x 1	10 mM Glycine-HCl, pH 1.5

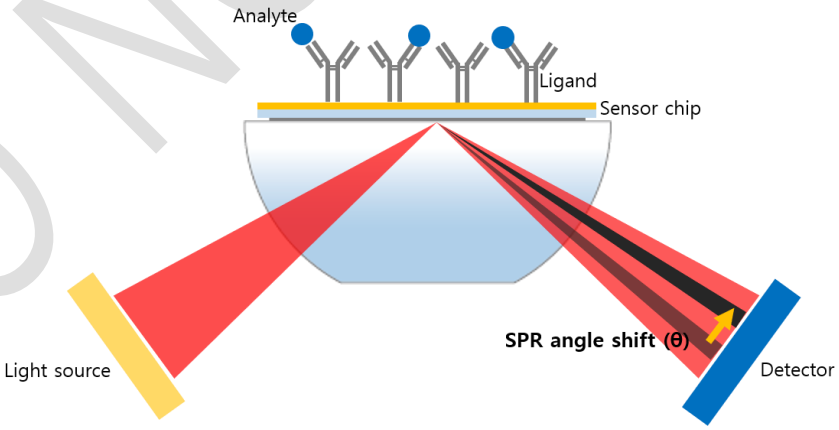
Backgrounds

This section describes about the terminology, the sensor chip introduction, immobilization techniques and analysis based on the theory used to analyze the molecular binding using iMSPR-Mini (SPR biosensors).

Basic concept

The basic concept of analyzing intermolecular bonds using iMSPR-Mini is to immobilize a ligand material (Ligand) on the surface of the sensor chip through covalent and non-covalent bonding techniques, and by passing an analyte dissolved in a solution (running buffer) through the immobilized ligand, a ligand-analyte binding reaction occurs, and the amount of binding is measured in real time for SPR signal change. (Figure 1)

The basic phenomena and measurement theory of SPR are not described in this handbook. Readers who need detailed explanations should refer to the postings on the blog (blog.naver.com/hipoo99) or the homepage (www.icluebio.com).



[Figure 1] Basic concept of intermolecular binding analysis using SPR biosensor

Unit of measurement (RU) and Real-time graph (Sensorgram)

Units of measurement are expressed in Resonance units (RU). This unit is proportional to the change in angle. In iMSPR-Mini device, it means 1000RU=0.1˚. A change in angle means a change in the mass of a surface or a change in the refractive index, that is, a molecule "bonded" or "dissociated" to the surface.

Sensorgram is a real-time graph that records (plot) changes in RU over time. Sensorgram allows researchers to observe the progress of molecular bonding.

COOH-Sensor chip

The sensor chip is deposited with a very thin thickness of Cr (adhesive layer, 2nm) and Au (resonant layer, 48nm) on a thin glass plate (0.3mm). And on the Au layer, a monomolecular layer with COO-terminals that can covalently bond R-NH2 using the amine coupling method is formed.

Ligand immobilization step

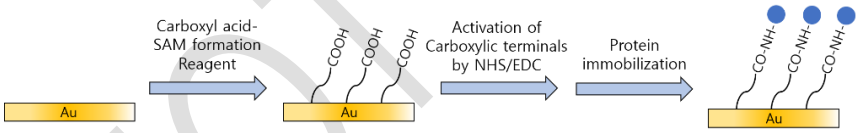
The binding of the ligand to the sensor chip surface is referred to as immobilization.

There are a wide variety of substances used as ligands such as antibodies, proteins, nucleic acids, aptamers, peptides, and chemicals. Depending on the characteristics of the ligand material (type of functional group, electrical charge, size and length, etc.), the surface function and immobilization method of the sensor chip can be determined.

In this handbook, the method of immobilization using amine coupling was described as a standard.

Amine coupling method

The amine coupling method is to covalently bond R_1-NH^2 and R_2-COOH to R_1-NH-CO-R_2. Proteins are mainly rich in amino acids with amine residues such as lysine, so this is a very frequently used method when using a protein as a ligand. A large amount of energy is required to directly covalently bond R_1(protein)- NH^2 to R_2(sensor chip)-COOH. Therefore, the reaction rate is very low, and the bonding is difficult. To accelerate the reaction, EDC (1-ethyl-3-(3-dymethylaminopropyl)carbodiimide) / NHS (N-hydroxysuccinimide) is used as an active substance. When performing this process, the surface of the sensor chip is changed to succinimide ester. Then, when R_1-NH^2 passes over the surface of the sensor chip, it becomes covalently immobilized on the surface very quickly. The succinimide ester, which remains after the ligand is immobilized, is deactivated using ethanolamine because the amine group of the analyte can be bound during analyte binding analysis.



[Figure 2] Amine coupling process]

Immobilization condition

When the ligand is immobilized using the amine coupling method, the concentration range of the ligand is generally 10~200 ug/ml. Depending on the characteristics of the ligand, the yield of immobilization on the surface of the sensor chip may be very low. The amine coupling method is basically ionic bonding. In other words, the immobilization yield increases when the ligand and the electrical charge characteristics of the surface are suitable for bonding.

It is the pH characteristics of the buffer that can suitably match the electrical charge characteristics of the ligand and the sensor chip surface. In order for the COOH-sensor chip to have a negative charge, it must be higher than pH

3.5. Therefore, a buffer with a pH of 3.5 or higher should be used for the immobilization buffer. At the same time, the ligand material must have a positive charge, so the pH condition of the immobilization buffer must be lower than the isoelectric point (pI) of the ligand. For example, if the ligand has a pI of 5.0, the suitable pH range for the immobilization buffer will be between 3.5 and 5.

The buffer mainly used as the immobilization buffer is sodium acetate buffer with an ionic strength of 10 mM or less.

Icluebio provides 5 mM sodium acetate buffer at pH 4.0/4.5/5.0/5.5 conditions.

For successful ligand immobilization, it is recommended to perform a pH scouting step to determine the pH conditions.

Immobilization levels

The binding capacity capable of binding analytes depends on how immobilized the ligand to the surface, i.e. the level of immobilization. The maximum analyte binding level (Rmax) depends on the level of immobilization of the ligand, and its theoretical calculation can be done as follows.

$$R_{max} = \frac{\text{Analyte MW}}{\text{Ligand MW}} \times \text{immobilized amount} \times \text{stoichiometric ratio}$$

Here, MW is the molecular weight, immobilized amount is the final RU value at which the ligand is immobilized, and stoichometric ratio is the ratio of how many analytes can be bound by one ligand.

The theoretical Rmax value assumes that the immobilized ligand has 100% activity, so the experimental Rmax is always lower than the theoretical value. This is because the degree of freedom of the ligand bound to the solid phase is reduced compared to the ligand that can move freely in the liquid phase.

The level of ligand immobilization should be determined according to the purpose of the experiment. If kinetics evaluation is the purpose, it is

normally advantageous to have a low level of immobilization, and for quantification purposes, it is generally advantageous to increase the level of immobilization.

Analysis of analyte binding

Run the running buffer using a pump on the sensor chip surface where the ligand is immobilized, and continue to flow until the signal is stable. This stable section at this time becomes the baseline in which only the ligand is immobilized on the surface. Then, the analyte dissolved solution is injected for a certain period of time using a pump. At this time, if the analyte binds to the ligand, the sensorgram will rise in real time. We mark this interval as the association level. And if only the running buffer is injected again, the RU value stops rising and the signal shows a tendency to stabilize or drop. This section is the dissociation phase.

The common reason for the signal rise in the association section is

① The analyte is bonded to the sensor chip surface

② The running buffer has a lower refractive index than the analyte dissolved buffer. (in the opposite case, the signal decreases.)

③ When the temperature of the experiment environment drops rapidly or the temperature of the buffer drops (when the temperature decreases, the refractive index increases and the signal rises, and when the temperature rises, the refractive index decreases and the signal decreases.)

The above three events can occur individually or simultaneously.

Among these events, the researcher would like to be in the case of ① only. Of course, a reference (or negative control) experiment can be conducted at the same time to remove the signal for its effect, but there are cases that cannot be eliminated even with such a control experiment, so the researcher must control the conditions so that cases ② and ③ do not occur.

In the case of ②, the effect can be minimized if the analyte is dissolved in

the running buffer used that day. In the case of ③, the temperature of the experiment environment must be controlled at a constant temperature, and the temperature must be adjusted by sufficiently exposing the buffer to the experiment environment before the experiment.

On the other hand, even in the case of ①, unwanted signals may be included. It is a signal generated when analyte, expressed as non-specific adsorption, is adsorbed to a non-ligand site on the surface of the sensor chip.

The icluebio COOH-sensor chip is designed to minimize non-specific adsorption. However, we do not guarantee that non-specific adsorption will not occur for all substances and all environments.

Therefore, first of all, researchers should pay attention to buffer composition and additional surface treatment to reduce non-specific adsorption.

If non-specific adsorption occurs despite these solutions, the signal for non-specific adsorption must be finally removed through a reference (control) experiment.

If the Analyte removes all nonspecific signals mentioned above in addition to the signal bound to the ligand, the stable RU value at the dissociatin stage can be determined as the level at which the analyte bound the ligand.

Regeneration

After performing an Analyte binding assay, you can analyze different concentrations of the same analyte, or analyze different analytes. At this time, it is possible to perform a new experiment by changing to a new sensor chip, but after removing the previously bound analyte, the new analyte binding analysis can be repeatedly performed on the same chip.

The step of desorbing only the analyte from the ligand so that a new analysis can be performed is called regeneration.

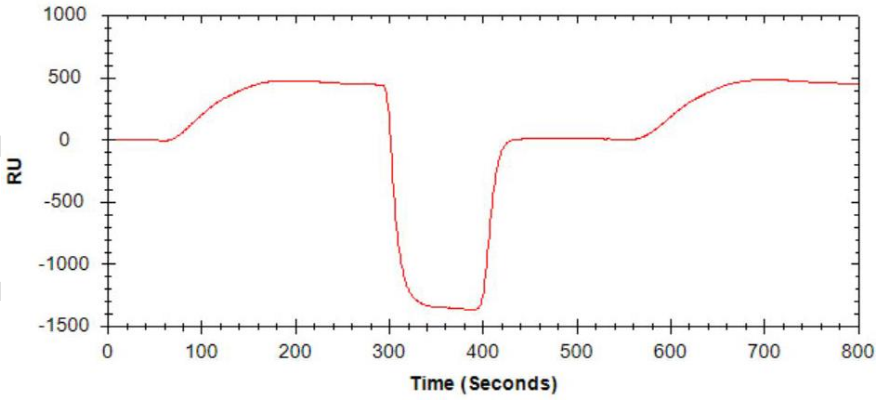
Ligand and analyte bonds are usually combined by a combination of hydrogen bonds and non-covalent bonds such as van der Waals attraction,

so the analyte can be removed from the ligand. Generally, reagents for removing analyte include acidic solutions (Glycine-HCl) or basic solutions (NaOH), solutions with high ionic strength (NaCl), and surfactants (SDS).

In addition to the complete removal of the analyte from the ligand during the regeneration phase, there is one important point. That is, after the analyte is removed, the function of the ligand must remain as it was originally. To do this, the analyte must be completely removed from the ligand under the mildest conditions possible.

Therefore, when conducting a new binding experiment, an experiment that optimizes the regeneration conditions must be preceded.

Optimization experiments should be conducted in the order of mild to strong conditions (e.g., in the case of glycine-HCl, proceed from pH 2.5, then proceed in the order of 2.0 and 1.5), and perform binding analysis of the same analyte of the same concentration by repeating at least 5 times. It is recommended to ensure that the analyte continues to be completely removed from the ligand and that the analyte continues to show the same binding level.



[Figure 3] regeneration process. If the same analyte is injected at the same concentration, the same binding level is shown in cycle 1 and cycle 2 as above.

Experimental practice using a basic training reagents set

This experiment is an example of a protein-protein binding assay. Ligand is protein A (MW: 20K), and analyte is human immunoglobulin G (MW: 150K) purified from serum.

Protein A specifically binds to the Fc region of hIgG. It is a protein that is mainly used for the isolation/purification of IgG type antibodies.

This is an example that is useful for screening and characterization studies in the development stage of antibody drugs and diagnostic antibodies.

In this chapter

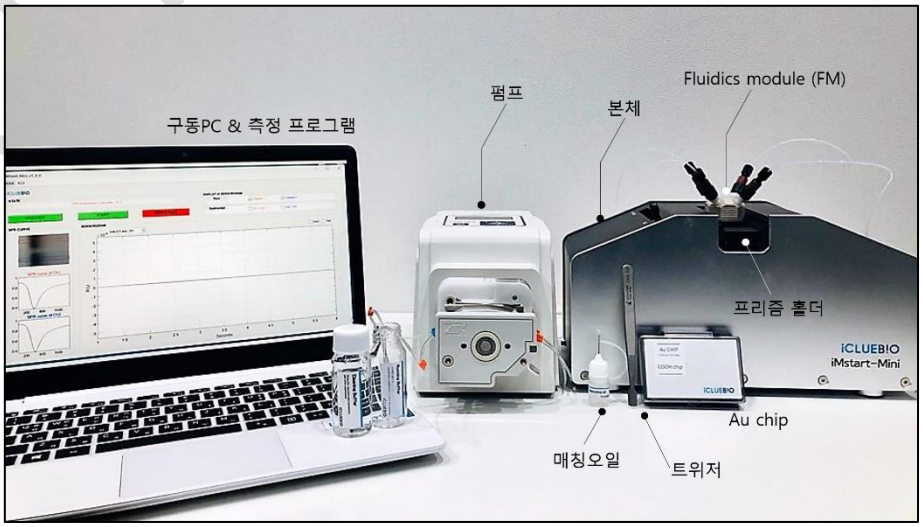
① device set up,

② protein A immobilization,

③ Five concentrations of hIgG binding analysis are sequentially described.

Device set up

The basic components of iMSPR-mini are shown below. In the photo, the names of the basic components are indicated. You can check the names of components named in the following descriptions by comparing them with photos.



Tubing connection for sample handling

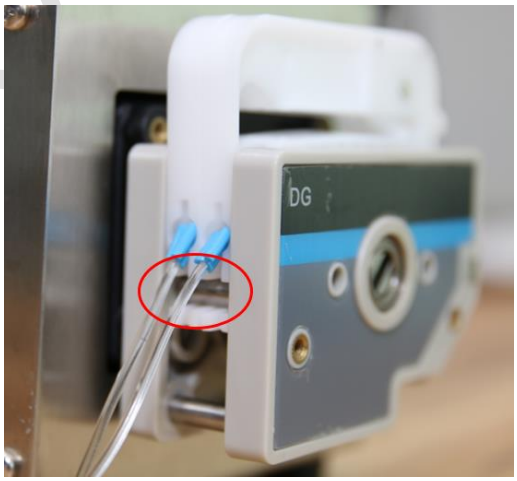
iMSPR-mini is a biosensor capable of observing the binding of analyte and ligand by passing the analyte in the liquid phase to the sensor chip (solid phase) on which the ligand is immobilized.

Therefore, you need a pump, a fluidic module, and tubing that connects the two devices for moving liquid samples to the sensor chip,. (If there is an optional bubble removal device, tubing to connect the three devices is required.)

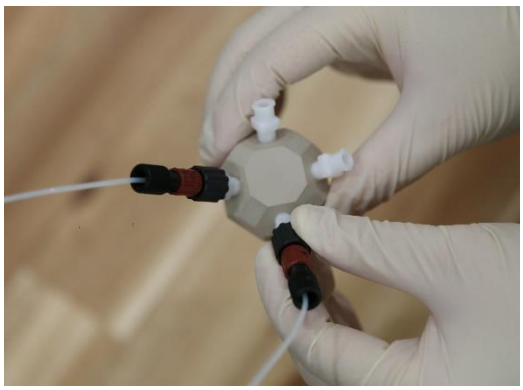
The iMSPR-mini is designed to allow users to easily connect tubing. Follow the instructions below to connect tubing.

1. Fix the 3-stop tubing to the two cassettes of the pump.

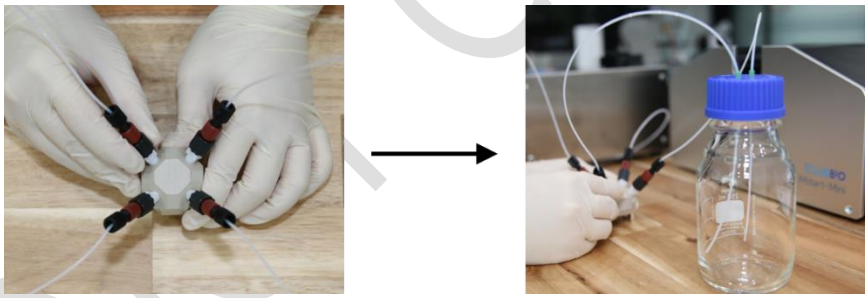
2. Install the cassette with fixed tubing on the pump head. The cassette should be caught in the pump head as shown in the red circle below.



3. Connect the fluidic module connection part of the tubing to the fluidic module.



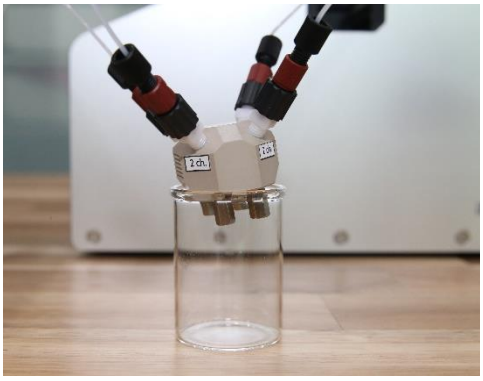
4. After connecting the fluidic module connection part of the waste tubing to the fluidic module, attach the opposite tubing to the waste chamber to complete the tubing connection.



Initial tubing cleaning (prime)

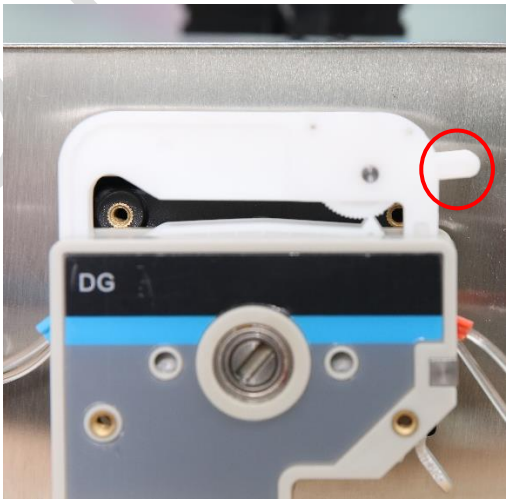
In this process, the tubing to the inlet is washed with a running buffer (1x HBST) and then filled with a buffer. Since it is not connected to the sensor chip yet, it proceeds at a fast flow rate. Proceed at constant flow rate for about 5 minutes.

1. Mount the fluidic module to the beaker

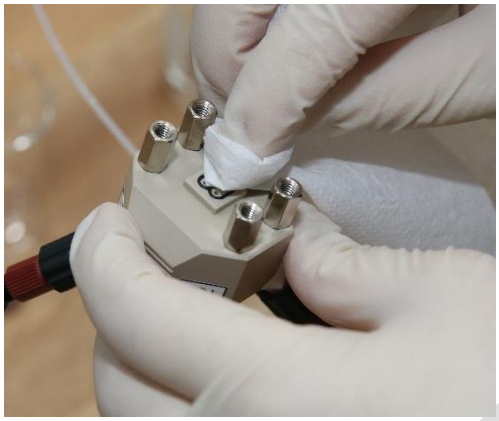


2. Soak the inlet tubing of the pump into the buffer chamber.

3. In the picture below, press the tubing to a suitable force by adjusting the area marked with a red circle on the cassette. After setting the flow rate of the pump to 100ul/min, press the start button (5 minutes).



4. After stopping by pressing the stop button, remove the remaining buffer in the channel part of the fluidic module.



Preparing sensor chip and combining with fluidic module

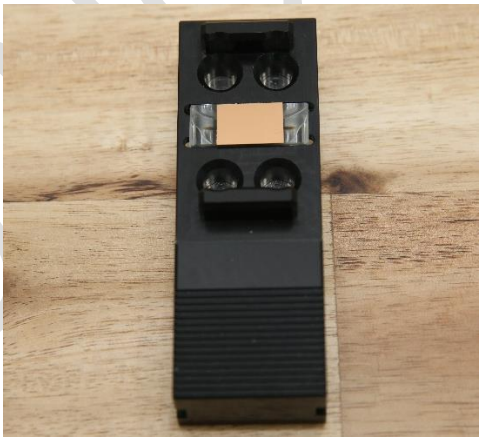
In iMSPR-mini, the prism is fixed to the prism holder, and the sensor chip is adhered to the prism using matching oil. At this time, the sensor chip must be completely bonded with matching oil to obtain correct results in the experiment.

1. Remove contaminants by wiping the prism with a microfiber cloth.

2. Drop the matching oil 1.5 ul (one drop) onto the prism.

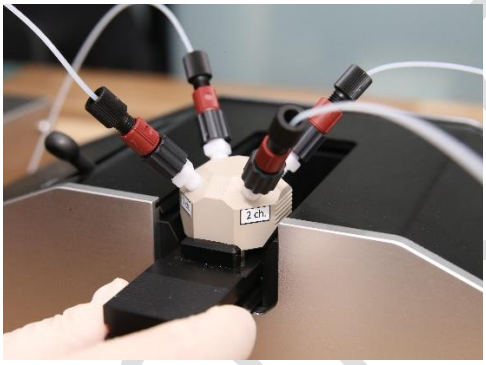
3. Combine the sensor chip on the prism.

4. Check for any abnormality in combining.



5. Combine fluidic module and prism holder.

6. Mount on the main device.

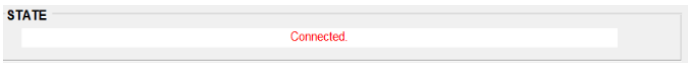


Software execution

Prepare to acquire the signal using the iMSPR-mini software.

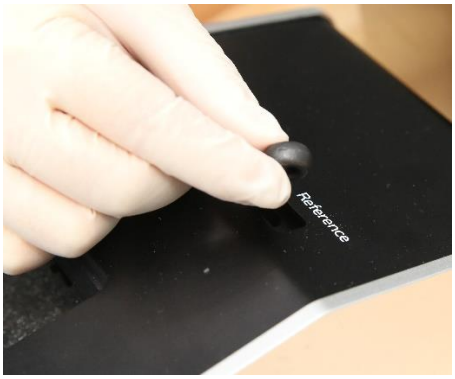
1. Connect iMSPR-min with PC
2. Execute software
3. Press the connect button in the STATE box for communication

4. Check the connection

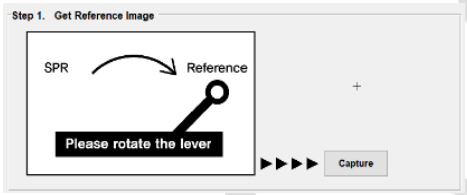


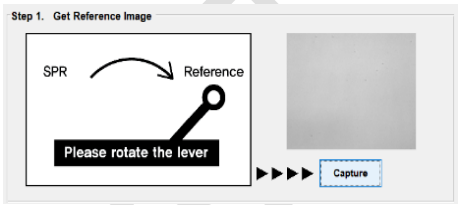
5. Press **Preview** button

6. Set polarization lever in reference direction



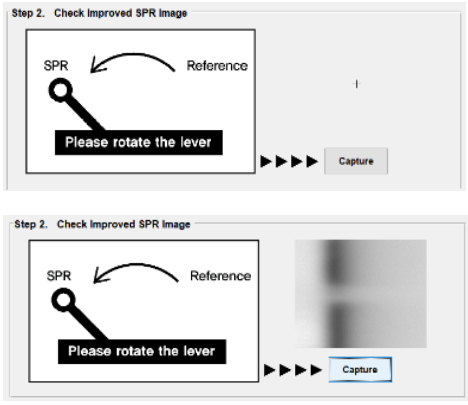
7. Click the Capture button in the Step1 section of the Image capture window.





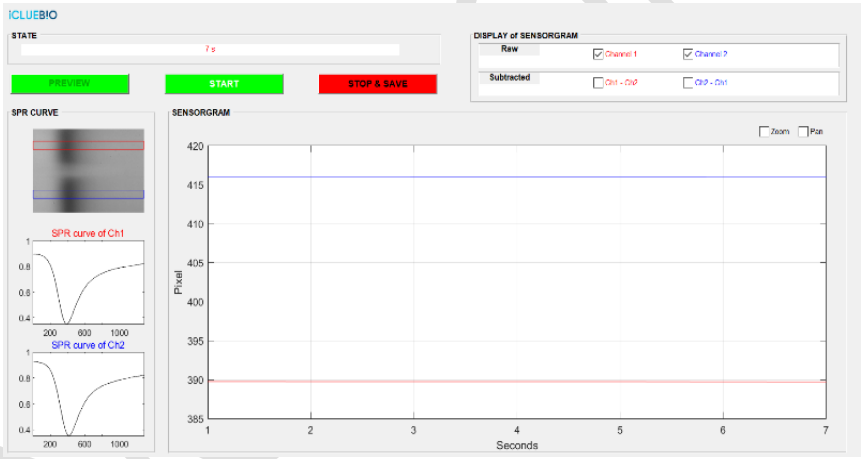
8. Set polarization lever in SPR direction

9. Click the Capture button in the Step2 section of the Image capture window.



10. Click the **confirm** button on the image capture window

11. Monitoring to check equilibrium

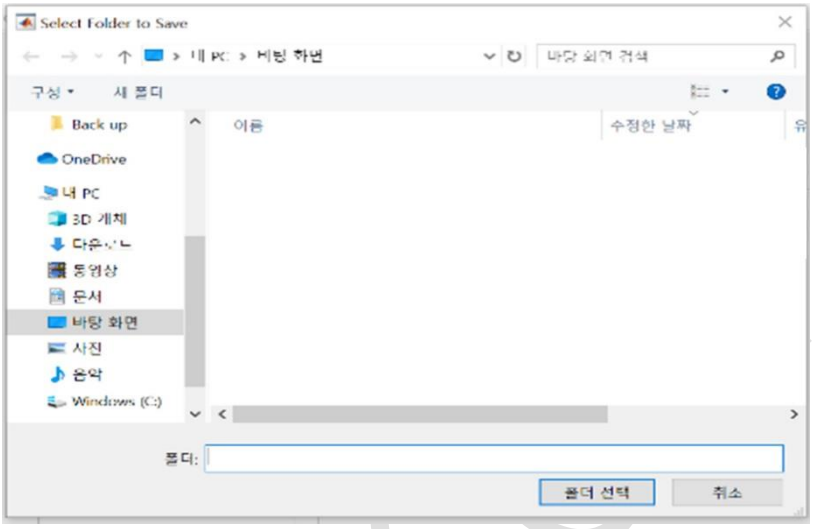


The real-time graph of both channels is checked until there is no significant fluctuation over time.

Check 1) The Y-axis value of the SPR curve must be about 0.4 or less.
Check 2) The difference between the pixel values of both channels of the sensorgram must be within 10 pixels.

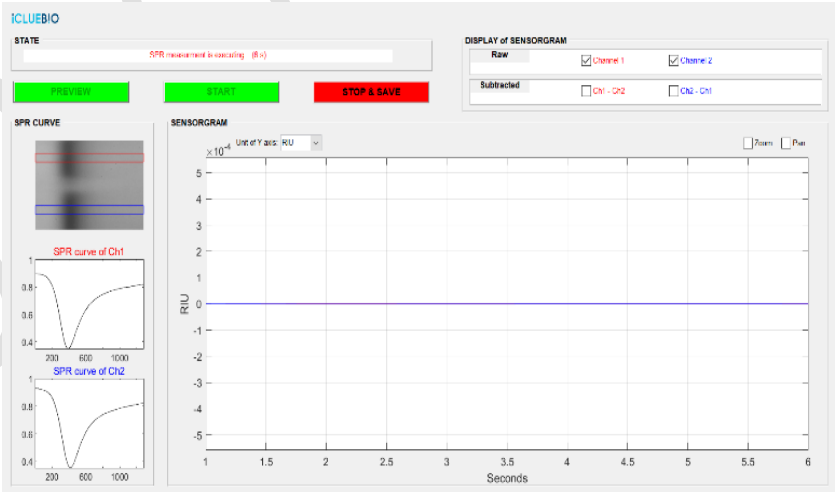
12. Press **START** button

13. Set the data storage path



After selecting a folder, click the Select Folder button determine the storage path.

14. Sensorgram acquisition



Signal acquisition starts at zero for both channels.

From now on, full-scale experiment will be conducted.

Ligand immobilization

This experiment is a ligand immobilization step for analyte analysis. Ligand is protein A.

The experiment proceeds according to the following procedure.

1. After setting the flow rate of the pump to 50ul/min, press the start button..

Activation

2. NHS and EDC are mixed in a 1:1 ratio of 250 ul each.
3. Stop the pump.
4. Soak the inlet of the two channel tubing into the NHS-EDC mixture tube.
5. Run the pump and inject the mixture for 4 minutes.
6. Stop the pump.
7. Transfer the tubing to the buffer chamber and run the pump for about 5 minutes.

Immobilization

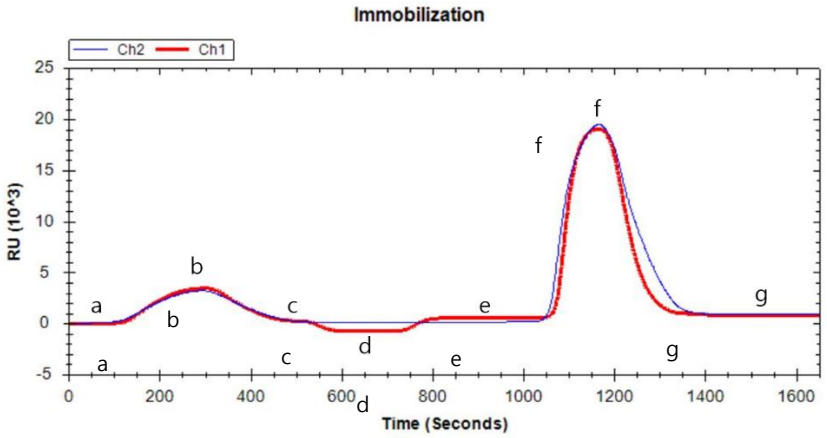
8. A diluted solution (50 ug/ml) was prepared by mixing 3 ul of a ligand (protein A) solution with 297 ul of 5 mM sodium acetate buffer. (Prepare during step 5)
9. Stop the pump.
10. Soak the channel 1 tubing inlet into the ligand diluent tube..
11. Run the pump and inject the diluted solution for 5 minutes.
12. Stop the pump.
13. Transfer the channel 1 tubing inlet to the buffer chamber and run the pump for about 5 minutes.

Blocking

14. Prepare 300 ul of a blocking solution.
15. Stop the pump.
16. Soak the tubing inlets for channels 1 and 2 into the blocking solution.
17. Run the pump and inject the blocking solution for 2 minutes.
18. Stop the pump.
19. Soak the channel 1 and 2 tubing inlets back into the buffer chamber
20. Run the pump for 10 minutes.
21. Click the Stop button of the software to save the result of ligand immobilization.
22. If you continue with the Analyte binding assay, the pump will continue to run.

Analysis of immobilization result

Channel 1: ligand channel (red line)



If you have successfully performed the above immobilization practice, you can obtain the graph as above.

a. Equilibrium state by buffer injection

b. RU rise by NHS/EDC

c. Equilibrium state by buffer washing, RU value higher than 'a' because NHS is combined

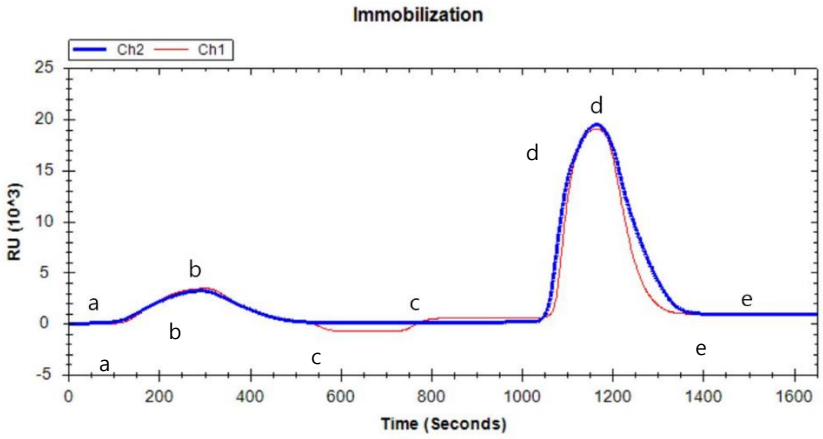
d. RU changes due to the ligand solution, and RU decreases initially due to the influence of an acetate buffer with a low refractive index, and RU increases as the amount of ligand immobilization increases.

e. Equilibrium state by buffer washing, RU value is higher than 'a' in proportion to the amount of ligand immobilization

f. RU rises by blocking solution

g. Equilibrium by buffer washing, usually at the same level as 'e'

Channel 2: reference channel (blue line)



If you have successfully performed the above immobilization practice, you can obtain the graph as above.

a. Equilibrium state by buffer injection

b. RU rise by NHS/EDC

c. Equilibrium state by buffer washing, RU value higher than 'a' because NHS is combined

d. RU rises by blocking solution

e. Equilibrium due to buffer washing, usually shows a slightly higher RU value than 'a'

Analysis of immobilization level (channel 1)

Immobilization RU = g(RU) - a(RU) = 60 – 0 = 60 RU

Theoretical Rmax prediction

$$R_{max} = \frac{\text{Analyte MW}}{\text{Ligand MW}} \times \text{immobilized amount} \times \text{stoichiometric ratio}$$

$$= \frac{150{,}000}{20{,}000} \times 60 \text{ RU} \times 1 = 450 \text{ RU}$$

The Rmax of the ligand and analyte information used in this experiment is expected to be about 3000 RU.

If it is an experiment for kinetics evaluation, set Rmax to 100 RU based on molecular weight of 20,000 Da.

Analysis of analyte binding

In this experiment, 5 concentrations of analyte are injected into the sensor chip to which the ligand is immobilized to analyze the degree of binding between the analyte and the ligand by concentration.

After experimenting by injecting one concentration, prepare for the next concentration experiment by performing the regeneration process. Repeat this process 5 times to practice.

Analyte injection and regeneration process is one unit analyte combined assay.

Channel 1 is the channel on which the ligand is immobilized, and channel 2 is the reference channel. Analyte binding assay runs both channels simultaneously and identically.

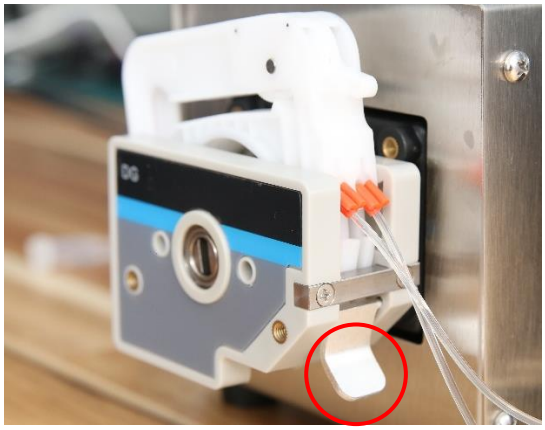
The unit analyte combination analysis practice is performed in the following steps.

1. Execute the Acquisition button on the software to start acquiring the signal.
2. Set the pump flow rate to 50 ul/min and press the start button.

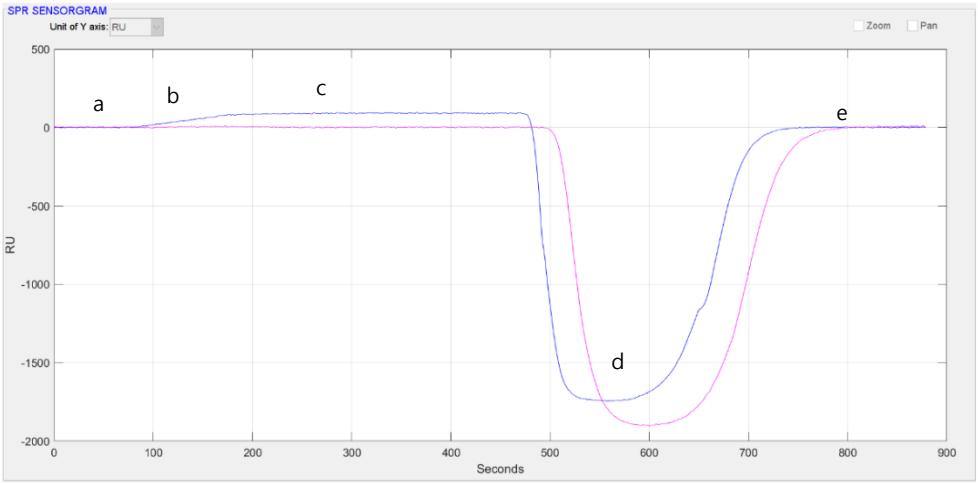
3. Flow running buffer until signal is stable.
4. Mix 6 ul of Analyte solution into 594 ul of running buffer.
5. Dilute the mixed solution 5 times at a ratio of 1/2 to prepare 300 ul (300 ul of sample + 300 ul of dilution buffer) for a total of five samples.
6. When the signal is stable, stop the pump and soak the two channel tubing inlets into the sample tube with the highest concentration.
7. Run the pump and allow to bind for 2 minutes.
8. After stopping the pump, move the tubing to the buffer chamber and run the pump again for 8 minutes.
9. After stopping the pump, transfer the tubing to the regeneration buffer tube and let it run for 2 minutes.
10. After stopping the pump, move the tubing to the buffer chamber and run the pump for 10 minutes.
11. Save the result by executing the Stop button on the software.
12. Repeat steps 1-11 for each concentration in the order of low concentration.

Finishing the experiment

13. 13. After replacing the running buffer with distilled water, run it for 30 minutes at a flow rate of 50 ul/min.
14. If not used for more than 1 week, 20% ethanol is flowed at the same flow rate for 10 minutes.
15. Remove the 3 stop tubing from the pump cassette by pushing the lever marked as red circle.



Analysis of analyte binding graph



a. Equilibrium state by buffer injection

b. Analyte and ligand binding section (association)

c. The dissociation of the analyte and the ligand is gradually stabilized by buffer washing.

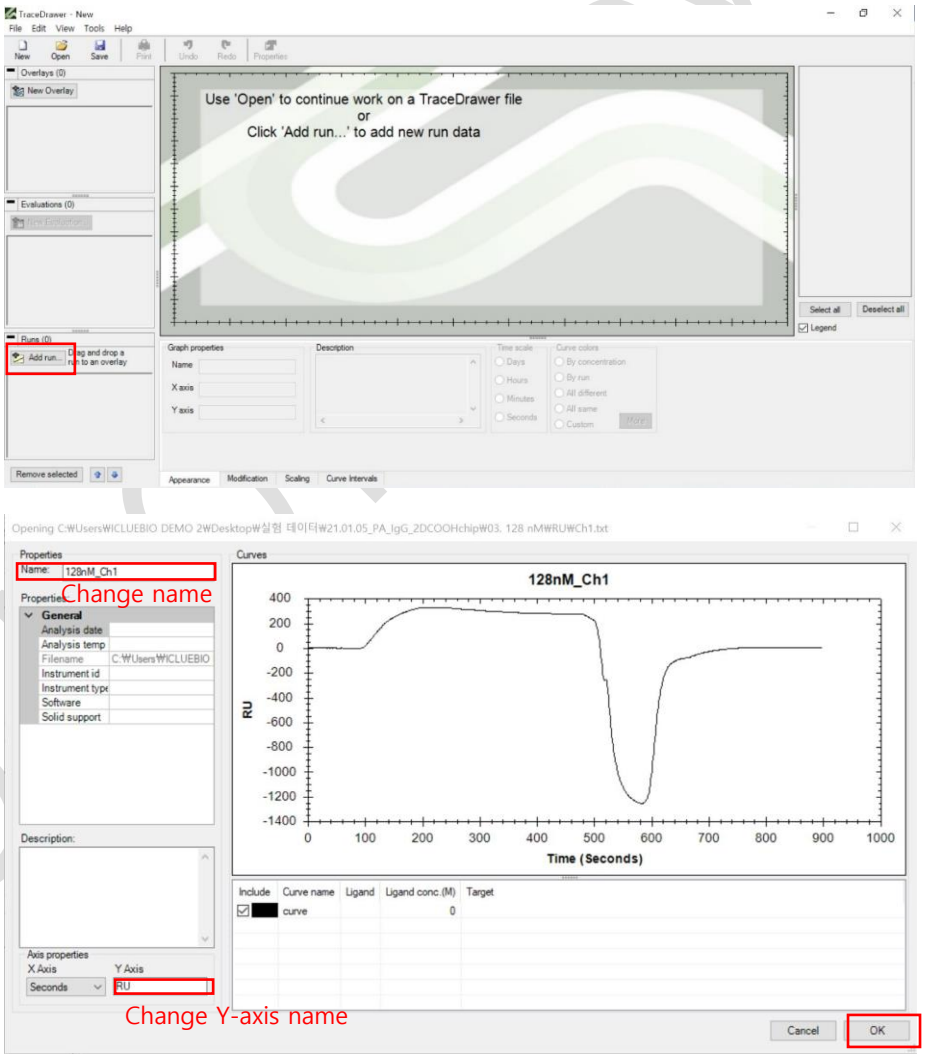
d. The section in which the analyte is completely dissociated by the regeneration buffer

e. The section in which the equilibrium again by a buffer wash

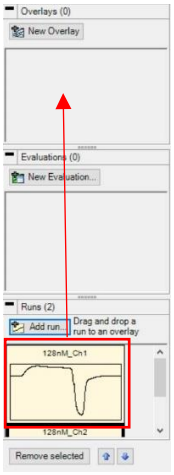
Kinetics evaluation

This chapter describes the process of analyzing kinetics values using the combined analysis graph of 5 concentrations acquired through practice and Tracedraw analysis software (sold separately).

1. Click Add run to load the text files (.txt) of the ligand channel (ch 1) and reference channel (ch 2) in the RU folder of the obtained data. In the window that appears, you can set the data name and y-axis name.

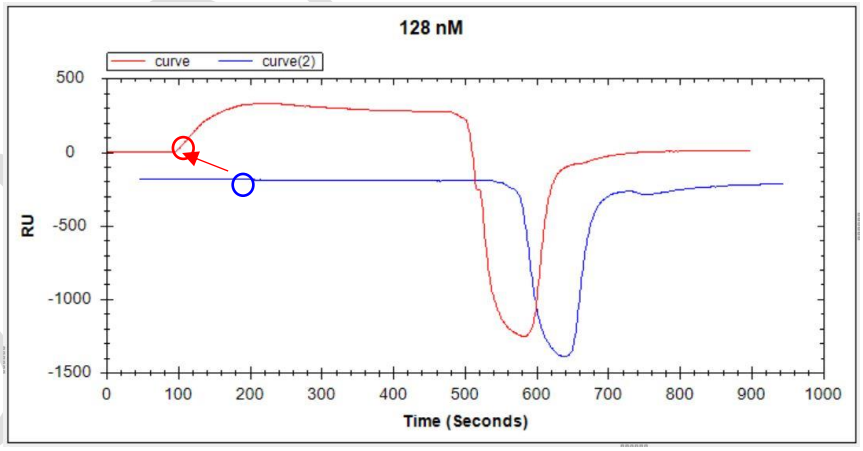


2. After creating a new overlay, drag the data of ch1 and ch2 contained in Runs and drop it on the overlay.

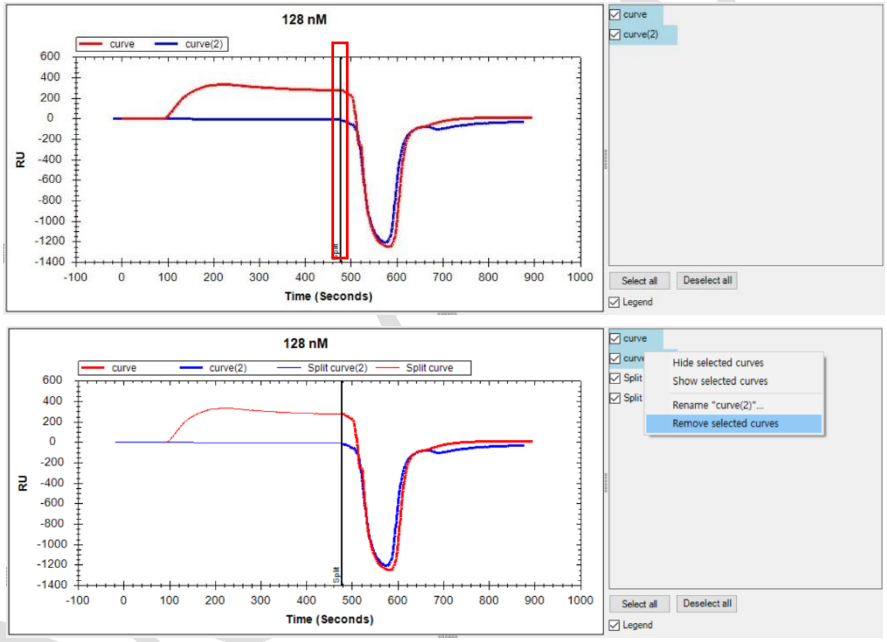


3. In the Appearance tab at the bottom, settings such as graph properties, time scale, and curve color can be changed.

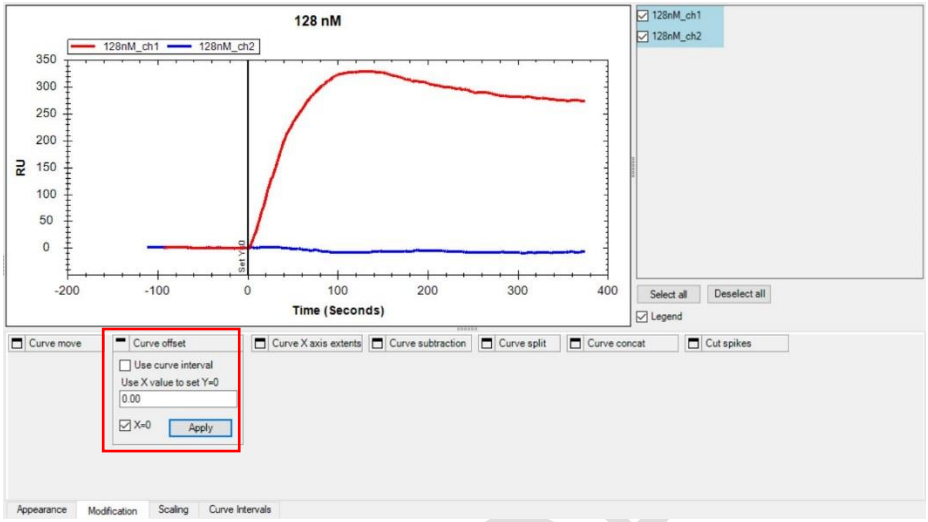
4. Move the curve using Alt + Left Click to make the start time of combining the two channels equal.



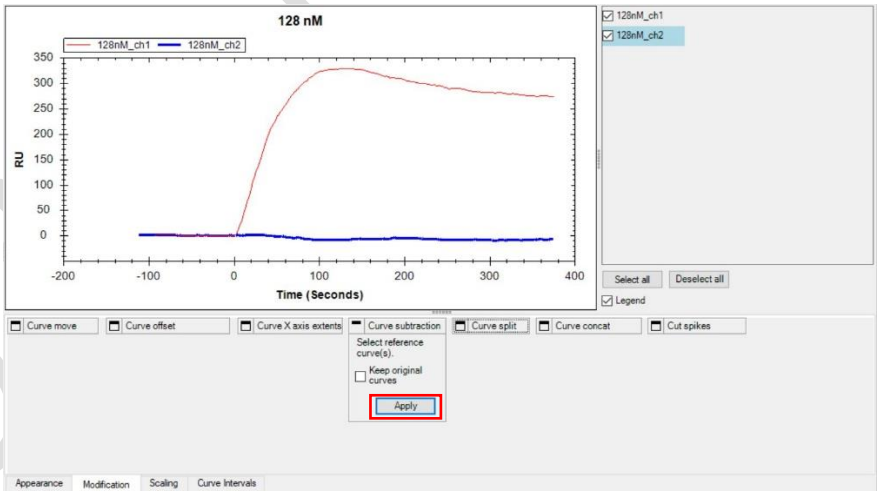
5. Select curve split in the modification tab at the bottom. After selecting both curves, move the split line to place it at the end of the curve's dissociation (the part just before regeneration) and cut it. The front curve required for kinetics evaluation is left, and the back part that is not needed is deleted by selecting the curve on the right side of the sensorgram, right-clicking, and clicking remove selected curve.

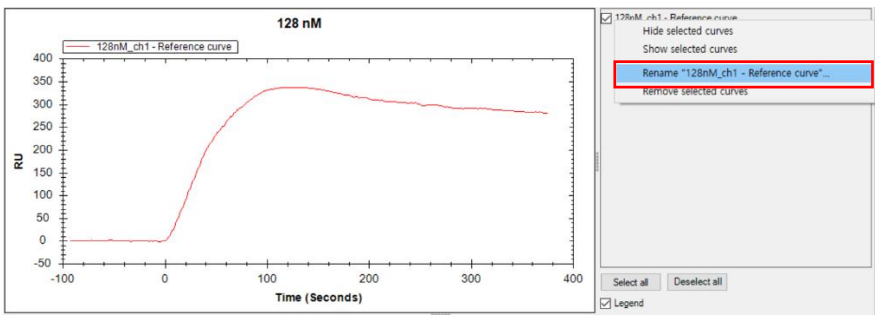


6. In the Modification tab, select the curve offset. With the two curves selected, align the line at the start point of combining, check x=0 and apply, then you can see that the start point of combining is set to (0,0).

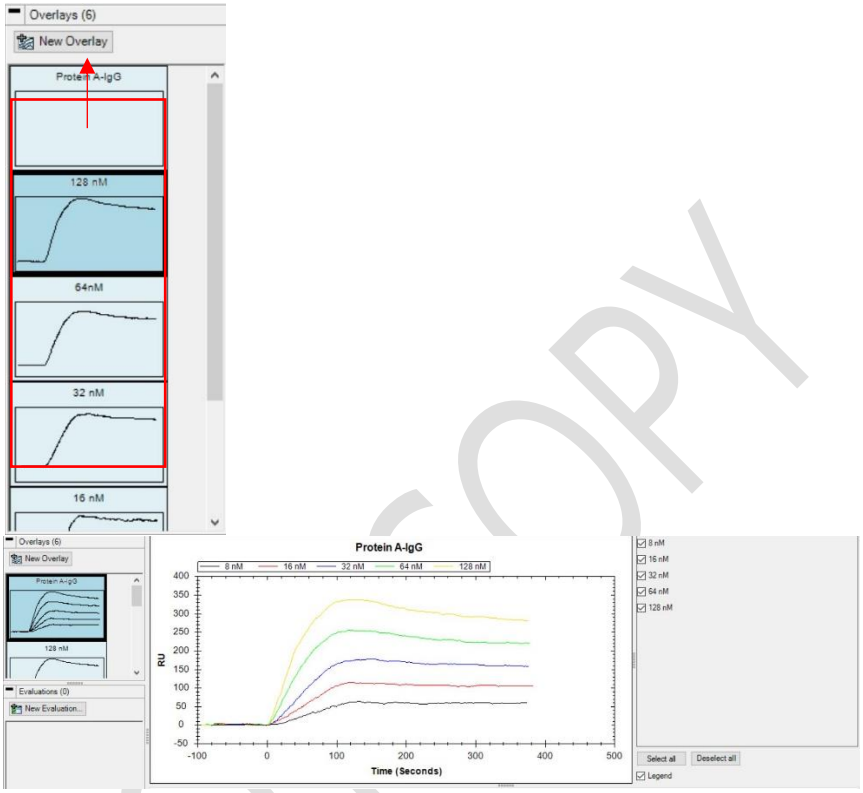


7. Click the curve subtraction on the Modification tab. When a reference channel is selected and applied, a curve is created by subtracting the reference channel from the ligand channel. At this time, you can right-click the curve name on the right to change it.

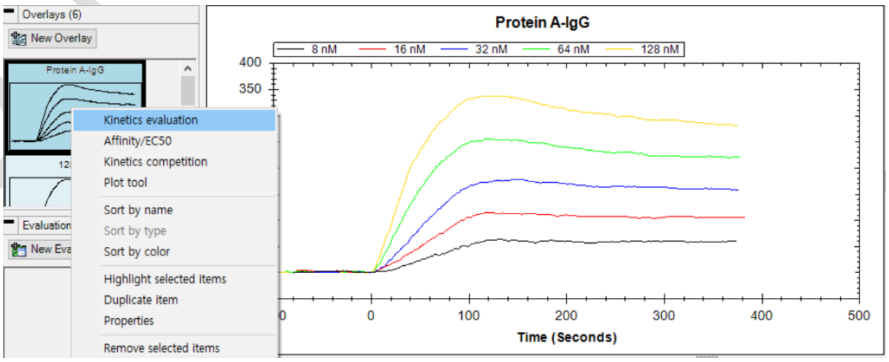




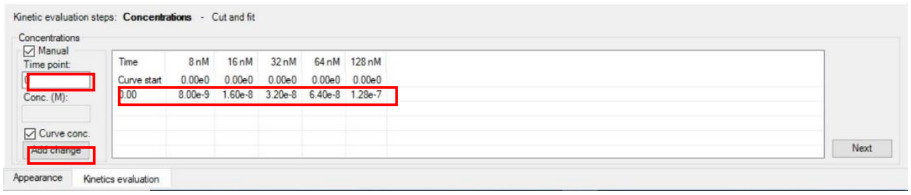
8. Steps 1-7 are run for each concentration. Drag the 5 created overlays and put them in one overlay.



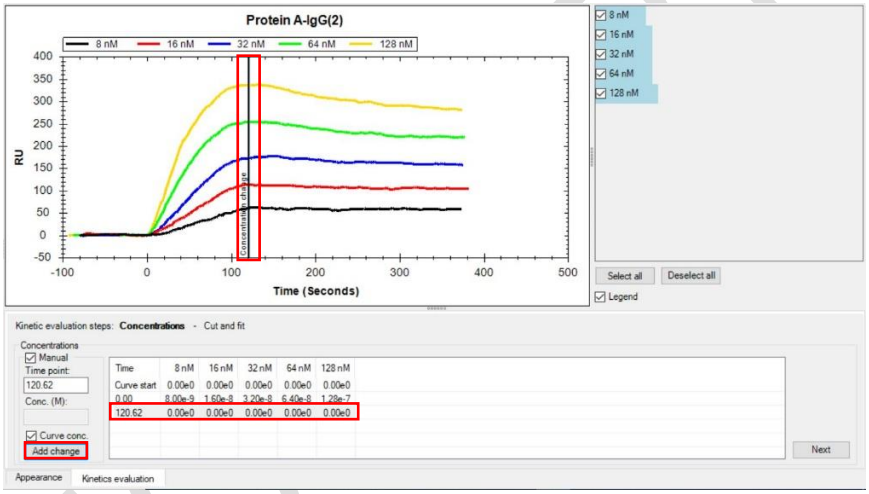
9. If you right-click the overlay thumbnail of five curves and select Kinetics evaluation, a new thumbnail is created in the evaluation tab.



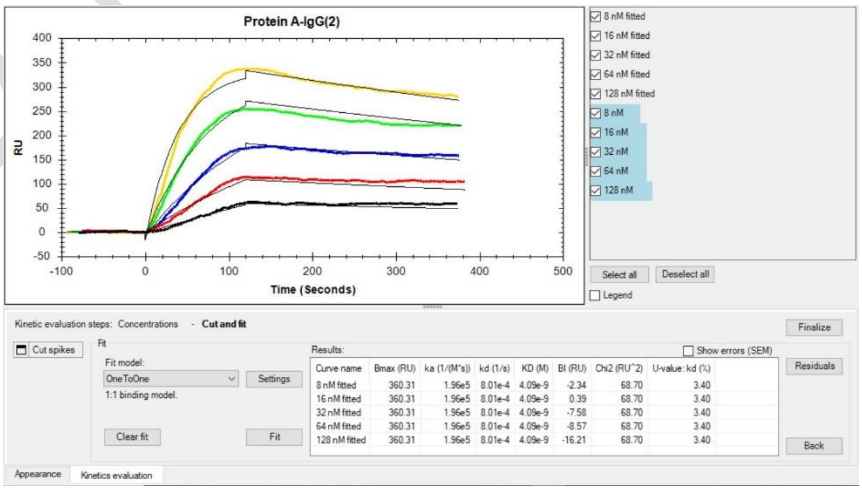
10. With all the curves selected, enter 0 at the time point at the bottom and click add change. Enter each concentration in the 0.00(time) row generated on the right. (ex. 128 nM is input as 128n)



11. Move the concentration change line of the graph to the highest point of the curve and click add change. Enter 0 for all concentrations in the new row created on the right.



12. Click Next and click Fit. Kinetic constants such as Ka, Kd, and KD can be obtained.



Warnings and device management

Caution for prism holder contamination

Be careful not to contaminate the prism mounted on the prism holder. Since light is incident and reflected by the prism and the signal is measured, contamination of the prism can cause problems in obtaining the correct signal.

If the surface where the light from the prism is incident is contaminated, wipe the contaminated area clean with a microfiber cloth.

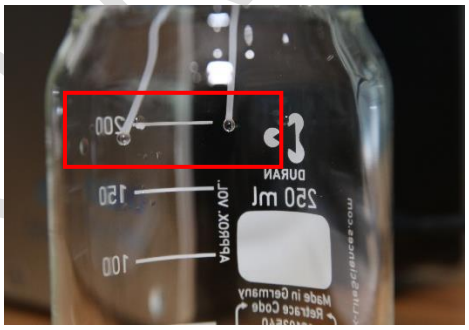
If there is any contamination that cannot be removed with a microfiber cleaner, please contact icluebio. (sales@icluebio.co.kr)

Caution for leakage of fluidic module-sensor chip joints

Since iMSPR-mini is a liquid based experiment, you should always be careful of leaks.

Use the pump flow rate below 100 μl/min.

When a liquid such as a buffer flows into the device after preparation for the experiment, make sure that the liquid flows correctly from the outlet.



Do not impact the fluidic module during use.

Tubing management

If the buffer remains in the tubing after the experiment is finished, the water

in the buffer is evaporated, and salt crystals may form in the tubing, causing clogging of the tubing. In addition, when stored for a long time, unwanted microorganisms may grow in the tubing, so tubing maintenance is required.

When performing the experiment again after storage for 1-2 days after ligand immobilization

In this case, it is important to maintain the activity of the ligand material, so continue to flow at a low flow rate (10 μl/min) until the moment you use an appropriate buffer capable of maintaining the ligand activity again. After ligand immobilization, it is recommended to complete the experiment as long as possible.

When performing the experiment again after storage for 1-2 days after the experiment is finished

In this case, after the experiment is over, replace the buffer with distilled water, flow it at a flow rate of 50 μl/min for about 30-60 minutes, stop the pump, store it, and remove the sensor chip that was used when the experiment was started again. , Install a new sensor chip to proceed with the experiment.

When the experiment is conducted on a weekly basis

In this case, after the experiment is over, the buffer is replaced with distilled water, flowed at a flow rate of 50 μl/min for about 30-60 minutes, and distilled water is replaced with 20% ethanol, and flowed at the same flow rate for about 30-60 minutes. Stop the pump and keep it as it is. Remove the sensor chip that was used when the experiment started again, and install a new sensor chip to proceed with the experiment.

When the experiment is conducted on a monthly basis

In this case, after the experiment is over, the buffer is replaced with distilled water, flowed at a flow rate of 50 μl/min for about 30-60 minutes, and distilled water is replaced with 20% ethanol, and flowed at the same flow rate for about 30-60 minutes. After removing the inlet to the outside so that air can be injected, flow it at the same flow rate for about 30 minutes, and then stop the pump after removing all liquid in the tubing.

Remove the sensor chip, wipe the matching oil with a microfiber cloth, and keep the prism holder and fluidic module separated.

Management for biomaterial desorption

Substances such as proteins may adsorb to the tubing over a long period of time. Therefore, it is recommended to perform cleaning for the desorption of biomaterials every 6 months.

Prepare the device in the same setting as for the experiment.

Apply 0.5% SDS to the tubing for at least 1 hour at a flow rate of 50 μl/min. After that, allow enough distilled water to flow for at least 1 hour at the same flow rate.

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