iCLUEBIO

User guide

Carboxyl modified Sensor chips &

Amine coupling kit

Products list

Sensor chip lists

Lists	ea/pkg	Product #	Recommended Analytes / Storage temperature
COOH-Au chip	10	PCCH1000	Proteins, Vesicles, Nano-particles, Cells, Peptides, Oligomers Storage: 2~8℃
C-Dex100 chip	3	DCCH1100	Proteins, Peptides, Oligomers Storage: -20℃
HC1000 chip	3	HCCH101KX	Small molecules Storage: -20℃

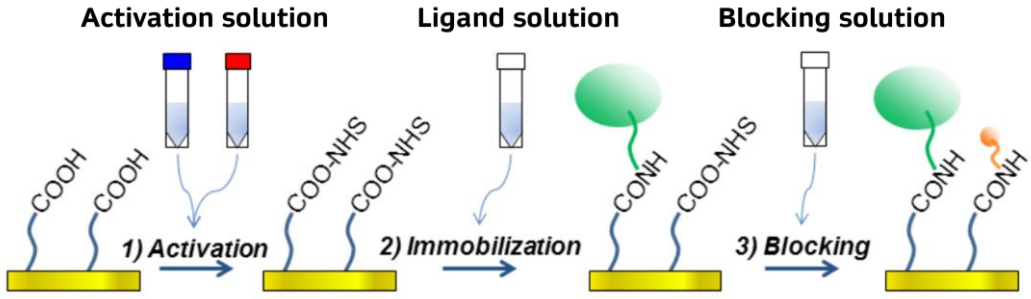
Buffers & Reagents

Lists	volume/pkg	Product #	Purpose of use
HBST buffer (10x)	50ml	RBHT1010-50	Running buffer, equilibrium, washing, dissociation
Amine coupling kit	100 tests	IMAM1000	Ligand immobilization
Glycine-HCl 50mM NaOH	50ml	RGGH10NNX	Regeneration

Note: For research only use

Amine coupling

Amine coupling is a method of covalently coupling an amine of a protein to a COOH sensor chip when the ligand is a protein. Since the protein is rich in amino acids with amine residues such as lysine and arginine, it can be very successfully immobilized on the COOH sensor chip. Since this immobilized ligand is covalently bonded, it is not desorbed during the regeneration step and has the advantage that it can be used continuously as long as the protein activity is not reduced. However, since the protein active site is immobilized on the surface of the sensor chip and the activity is sometimes reduced, it is recommended to select another immobilization method if no signal is seen even in the positive sample. (If the protein has a tag, the protein can be directionally immobilized by non-covalent specific binding.)



Amine coupling kit lists

Products #	Including
IMAM1000	EDC 1 g (Powder) NHS solution, 25 ml Blocking solution, 30 ml 1M NaCl borate buffer, 50 ml Acetate buffer, 25 ml 10X HBST, 50 ml

Amine coupling kit: Preparation and Storage

Contents	Aliquots and storage
EDC 1 g (Powder)	1. Dissolve in 25 ml of DIw 2. Aliquots in 150 ul each 3. Storage: -20℃
NHS solution, 25 ml	1. Aliquots in 150 ul each 2. Storage: -20℃
Blocking solution, 30 ml	1. Aliquots in 250 ul each 2. Storage: -20℃
Acetate buffer, 25 ml	1. Aliquots in 250 ul each 2. Storage: 2~8℃
1M NaCl borate buffer, 50 ml	Storage: room temperature
10X HBST, 50 ml	Storage: room temperature

Sensor chip loading & priming

1 Carefully take out the refrigerated sensor chip from the sensor chip case using a flat tweezer and mount it in the prism holder.

Note: The side that is attached to the sensor chip case is the side that connects with the prism of the prism holder. Reversing the sensor chip does not guarantee 100% performance for reuse.

2 After priming with 1X HBST buffer, wash the sensor chip with 1M NaCl borate buffer for 3-5 minutes.

Note: Prepare 10X HBST buffer by diluting 10-fold in DIw just before the experiment.

Ligand immobilization

1 Prepare frozen or refrigerated EDC (150 ul), NHS (150 ul), Blocking solution (250 ul) and regeneration buffer at room temperature.

Note: Ligand materials are prepared according to material properties. After preparing at room temperature, proceed with the experiment as quickly as possible.

2 Activation: After preparing 300 ul by mixing EDC and NHS 1:1, flow on the sensor chip for 5-10 minutes to make the COOH surface the NHS surface.

Note: The recommended flow rate for the activation step is 10-30 ul/min.

3 Ligand immobilization: Dilute ligand in acetate buffer to a concentration of 1 ~ 100 ug/ml and inject for 1 ~ 30 minutes.

Note: The immobilization step is performed by adjusting the time, flow rate, and concentration according to your target immobilization level.

4 Blocking: Inject blocking solution for 5 minutes (flow rate 30 ul/min).

Note: If there is a lot of nonspecific adsorption as a result of binding to analyte, prepare serum albumin at 100 ug/ml and perform additionally before the blocking step.

5 Pre regeneration: In order to desorb the non-covalently adsorbed ligand, the regeneration buffer is applied 1 to 5 times for 3 minutes (flow rate 50 ul/min). The number of runs is determined by monitoring ligand desorption.

Analytes interaction

1 If no more desorbed signals are observed and sensorgram equilibrium is observed, analyze analyte binding. First, inject 1 to 3 representative concentrations (100 nM, 1 uM, 10 uM) to check if there is a signal.

Note: For chemical, it is recommended to perform up to 100 uM.

2 If a signal is detected, dilute by 2 times from the concentration determined to be the saturation concentration, and obtain 5 or more sensorgrams for each concentration. A concentration of 0 nM (running buffer) can also be used to obtain a sensorgram and correct for non-binding signals.

Note: For kinetics evaluation, it is recommended to proceed with a flow rate of 50 ~ 70 ul/min. For bonds that do not dissociate well, it is recommended that the dissociation period be 5 to 10 times longer than the association period.

Regeneration

For bonds that do not dissociate completely, a regeneration step using a regeneration buffer is required. To analyze multiple concentrations, regeneration is performed between concentrations. Glycine-HCl pH2.5 solution is evaluated first, and if regeneration is not completely achieved, change to pH1.5 solution. If regeneration does not work well with a glycine-based solution, change to NaOH.

Ligand immobilized chip storage

The sensor chip to which the ligand is immobilized can be stored using the storage kit provided by iclubio. After attaching the dedicated storage FM to the prism holder, load the glycerol buffer.

Note:

1. For more detailed instructions, please refer to the handbook provided when purchasing the device.
2. Application Note 1, 2, 3, and 4 provided at www.icluebio.com are all written based on the COOH sensor chip. Please refer to the experimental method.

www.icluebio.com

icluebio's sensor chips, buffers, and reagents are manufactured in Korea, and are finally delivered to customers through precise quality inspection by a specialist.

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