CD Bioparticles

Amine Coupling Kit

Catalog Number: DNG-SAM01

Please read this instruction manual carefully before using the product

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1. Description

Amine coupling is one of the most common methods to immobilize a protein or a small molecule containing primary amine group through a covalent bond on biosensors used in QCM (Quartz Crystal Microbalance), SPR(Surface Plasmon Resonance) or an electrode analysis. Amine coupling reaction allows to activate a carboxylic group to amine reactive function where a compound containing amine group binds.

Amine Coupling Kit contains all necessary reagents and buffer solutions for activation of carboxylic acid, immobilization of protein, and blocking of residual activated ester. The NHS/WSC solution is the activating solution that is easily prepared because each NHS and WSC is subdivided into tubes that only require reconstituting with provided buffer. When 0.2 ml of NHS/WSC solution is used for activation, this kit is sufficient to immobilize about 40 protein samples.

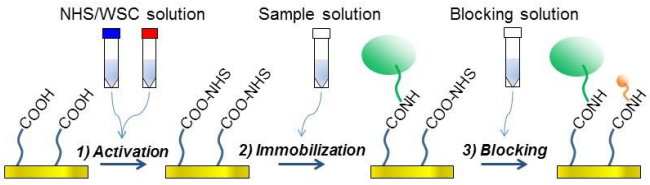

Fig1. Illustration of Amine Coupling Kit

2. Kit Components

WSC	x 4
NHS	x 4
Activation buffer	20 ml x 1
Reaction buffer	10 ml x 1
Blocking solution	20 ml x 1

3. Required Equipments

- Micropipette (1,000 µl)
- Microtube
- Carboxylic acid-containing substrate
- PBS

4. Preparation of Solutions

100 mmol/l WSC solution

Add 1 ml Activation buffer to a WSC tube, and pipette to dissolve.

*Aliquot the solution and store at -20°C. The solution is stable for 2 months.

100 mmol/l NHS solution

Add 1 ml Activation buffer to a NHS tube, and pipette to dissolve.

*Aliquot the solution and store at -20°C. The solution is stable for 2 months.

Sample solution

*For protein

Prepare 10-100 µg/ml of protein solution with Activation buffer. Please note the suitable concentration to prepare an appropriate biosensor depends on a protein to be immobilized.

*For small molecule

Prepare 1 mg/ml solution with Reaction buffer.

5. General Protocol

Batch method

1.Mix 200 µl of 100 mmol/l WSC solution and 200 µl of 100 mmol/l NHS solution by pipetting in a microtube.a)

2.Apply the mixed solution onto the surface of carboxylic acid containing substrate, and leave it at room temperature for 10 minutes.

3.Wash the activated substrate with PBS.

4.Apply sample solution to the surface of the activated substrate, and leave it at room temperature for 30 minutes.

5.Wash the immobilized substrate with PBS.

6.Apply Blocking solution to the surface, and leave it at room temperature for 30 minutes to block residual activated esters.

7.Wash the substrate with PBS.

Flow method

Set a substrate on an instrument. Set the flow rate and running time of the instrument at 10 µl/min and 10 min, respectively.

1.Inject PBS to equilibrate the surface of the substrate.

2.Mix 200 µl of 100 mmol/l WSC solution and 200 µl of 100 mmol/l NHS solution by pipetting in a microtube.a)

3.Inject 100 µl of the mixed solution.

4.Inject PBS and equilibrate the signal.

5.Inject 100 µl of sample solution.

6.Inject PBS and equilibrate the signal.

7.Inject 100 µl of Blocking solution.

8.Flow PBS until the signal is equilibrate.

*The mixed solution is not stable. Use the solution immediately after the preparation.

6. Storage

Store at 0-5°C.