NTA-SAM Formation Reagent

Cat. No: DNG-SAM04

DESCRIPTION

Description

Combination of His-tagged proteins (Histidine-tagged proteins) and Ni-NTA (nickel-chelated nitrilotriacetate) is one of the techniques to immobilize proteins through a Ni-NTA SAM (self-assembled monolayer) formed on a gold-coated substrate, which can be utilized for QCM (quartz crystal microbalance) or SPR (surface plasmon resonance) measurement.

The reagent solution for NTA-SAM formation on a gold-coated substrate can be easily prepared by dissolving NTA-SAM-Formation reagent with ethanol, and prepared Ni-NTA-SAM can immobilize His-tagged protein efficiently.

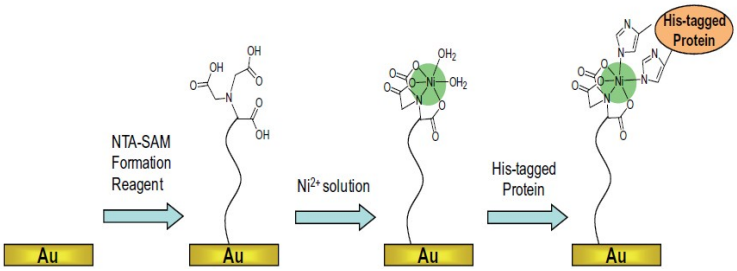

Fig1. Illustration of NTA-SAM Formation Reagent

GENERAL PROTOCOL

General Protocol

1) Add 1 ml ethanol to the tube and dissolve the reagent by pipetting to prepare 2 mmol/l NTA-SAM solution [a)]. Then, dilute the solution 10-fold with ethanol for Step 2).
2) Clean the gold surface of a substrate with Piranha solution [b)] prior to preparation of NTA-SAM. Immerse the substrate in the reagent solution prepared at Step 1) at room temperature and leave it over night [c)].
3) Wash the substrate several times with ethanol and purified water sequentially.

a) If the reagent does not dissolve by pipetting, use an ultrasonic bath or vortex. Use the freshly prepared reagent solution at Step 2).

b) Mix 3 volumes of concentrated sulfuric acid and one volume of the hydrogen peroxide solution to prepare a Piranha solution. Handle with great care; a Piranha solution is highly corrosive.

c) Store the SAM-coated substrate under nitrogen gas in a tightly sealed glass container at 0-5ºC.

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EXPERIMENTAL EXAMPLE

Experimental Example

Monitoring of protein binding processes by QCM.

1) Wash the NTA-SAM-formed substrate with 1 mmol/l NaOH solution.
2) Immerse the substrate in 40 mmol/l NiSO4* solution prepared with purified water at room temperature and leave it for 1 hr, and then wash several times with 150 mmol/l NaCl solution and purified water.
3) Set the substrate to a QCM instrument according to the manufacturer's instruction, and add 450 ml HBS (10 mmol/l HEPES-buffered saline, pH7.5) to the sensor cell for about 30 minutes.
4) After equilibration, add 10 ml of 5 mg/ml His-tagged Protein A solution in HBS and monitor the frequency modulation. (A)
   *NiSO4 is not included in this product.

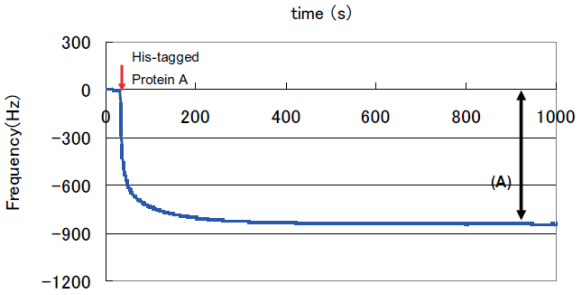

Fig.2 Frequency modulation during protein binding processes monitored by QCM.
(A) A large frequency drop is observed due to the His-tagged Protein A binding onto the surface.

Monitoring of protein binding processes by SPR.

1) Set the NTA-SAM formed substrate to a SPR instrument according to the manufacturer's instruction, and run HBS (10 mmol/l HEPES-buffered saline, pH7.5) containing 0.05 % TWEEN 20 on the substrate at flow rate 0.1 ml/min for 30 minutes.
2) After equilibration, run 40 mmol/l NiSO4* solution for 5 minutes, and then the substrate is washed with HBS containing 0.05 % TWEEN 20.
3) Run 0.1 mg/ml His-tagged Protein A solution prepared with HBS containing 0.05 % TWEEN 20 for 1 minute. Immobilization process of the Protein A to the Ni-NTA surface is monitored (A), and then wash the substrate with HBS containing 0.05 % TWEEN 20.
4) Run 0.1 mg/ml Rabbit IgG solution prepared with HBS containing 0.05 % TWEEN 20 for 1 minute. Immobilization process of the Rabbit IgG to the Protein A is monitored (B), and then, the substrate is washed with HBS containing 0.05 % TWEEN 20.
   *NiSO4 is not included in this product.

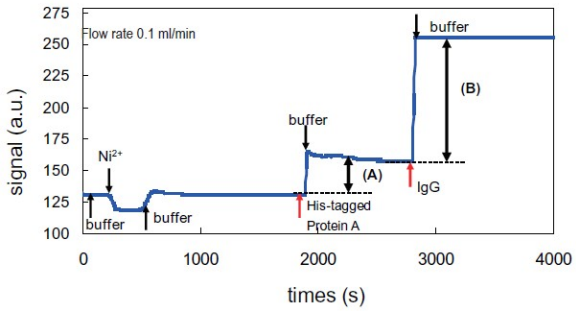

Fig.3 Intensity modulation during protein binding processes monitored by SPR.
(A) SPR-Intensity is increased by His-tagged Protein A binding to the Ni-NTA surface.
(B) SPR-Intensity is increased by Rabbit IgG binding to the His-tagged Protein A.

STORAGE AND SHIPPING

Storage	Store at 0-5ºC.

Note

Note	Since a trace amount of colorless or slightly yellowish liquid is in the tube, please centrifuge prior to use.

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