Biotin-SAM Formation Reagent

General Information

Various methods are available to immobilize proteins on a gold surface through self-assembled monolayers (SAMs) to create biosensors used in a QCM (Quartz Crystal Microbalance) or a SPR (Surface Plasmon Resonance) such as to make a covalent bond with proteins through an activated carboxylic group on the SAM surface, to fix Histidine-tagged protein (His-tag protein) through a Ni-NTA chelate on the surface, and to fix biotinylated protein through a streptavidin molecule on SAM surfaces. Biotin-avidin method is the most popular system to immobilize protein on a sensor due to the quick and very stable binding. Biotin-SAM Formation Reagent is specifically designed to prepare avidin-coated biosensors using streptavidin or NeutrAvidin. The biotinylated-surface prepared with this reagent can hold more streptavidin than conventional biotin-SAM surfaces. Additionally, the streptavidin surfaces prepared with this reagent can minimize non-specific protein adsorption.

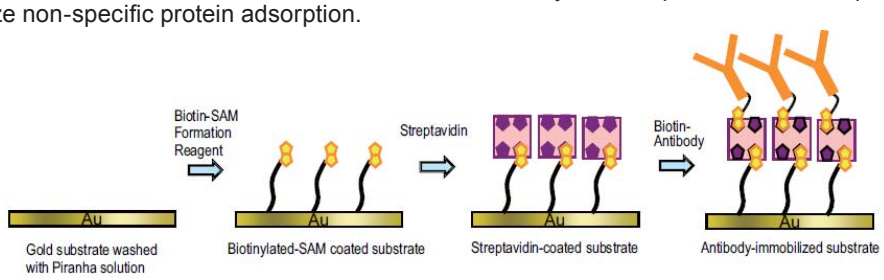


Fig.1 Preparation of a biosensor using Biotin-SAM Formation Reagent and streptavidin.

Contents

1 µmol x 3

Storage

Store at 0-5ºC.

Biotin-SAM General Protocol

Preparation of a Biotin-SAM surface on a gold substrate

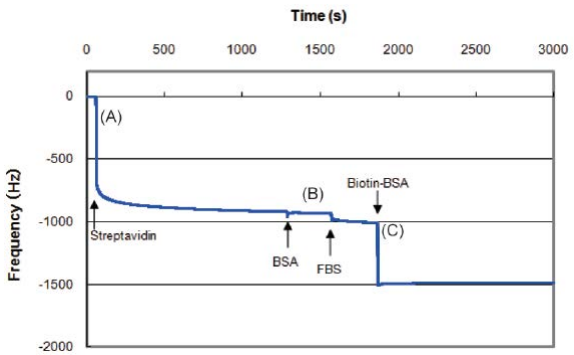
1) Add 1 ml ethanol to a tube, and pipette to dissolve the reagent to prepare 1 mmol/l Biotin-SAM solution.[a)] Then, dilute the solution 10-fold with ethanol for Step 2).
2) Clean a gold surface of a substrate with Piranha solution [b)] prior to preparing Biotin-SAM. Immerse the substrate in the reagent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

a) If the reagent does not dissolve by pipetting, use an ultrasonic bath or warm the tube around 40ºC. Use freshly prepared reagent solution at Step 2).
b) Mix 3 volumes of sulfuric acid and one volume of hydrogen peroxide solution to prepare Piranha solution. Since Piranha solution is severely caustic, handle with great care.
c) Store the SAM-coated substrate under nitrogen gas in a tightly sealed glass container at 0-5ºC.

Experimental Example

Monitoring of protein binding processes on the biotin-SAM surface with QCM.

1) The Biotin-SAM-coated substrate was attached to a cell of a QCM instrument and set the cell on the instrument according to the manufacturer's manual.
2) Four hundred fifty µl PBS was added to the cell. After the stabilization of the frequency, 20 µl streptavidin solution (10 mg streptavidin dissolved with 1 ml PBS) was applied.[A)]
3) After the stabilization of the frequency, frequency was monitored to evaluate non-specific binding of BSA (bovine serum albumin) and FBS (fatal bovine serum) by adding 8 µl BSA solution (10 mg BSA dissolved with 1 ml PBS) and then by adding 8 µl FBS solution (10 mg FBS dissolved with 1 ml PBS).[B)]
4) The solution was removed and the cell was washed with PBS several times.
5) Four hundred fifty µl PBS was added to the cell.
6) After the stabilization of the frequency, 8 µl Biotin-BSA solution (12 mg Biotin-BSA dissolved with 1 ml PBS) was applied.[C)]
7) The frequency was measured to monitor an immobilization of Biotin-BSA to the streptavidin-coated substrate.



Notes)

Data was prepared by AFFINIXQNµ (Initium).

A) A large decrease in frequency was observed due to the streptavidin binding on the surface.
B) Very slight frequency change was caused by non-specific FBS binding.
C) A large and immediate change in frequency was observed by Biotin-BSA binding on the surface.

Streptavidin surface prepared with Biotin-SAM Formation Reagent minimize the unspecific binding of undesired protein such as BSA or FBS. Since the cell was washed at step 4), the data at step 4) is left out.

Fig. 2 Frequency monitoring during protein binding processes by QCM instrument.