TEXAS INSTRUMENTS

APPLICATION BRIEF #002

Spreeta™

Immobilization of Ligand: Method 2

Avidin-biotin Ligand Immobilization

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Immobilization of Ligand: Method 2

ABSTRACT

In this method the ligand is linked to the gold surface using the avidin-biotin affinity system.

Introduction

As in Immobilization Method 1, here we preserve the binding activity of the ligand by linking it to a protein film. However, in this method neutravidin is captured onto a physisorbed film of biotinylated BSA. The ligand is then biotinylated and affinity captured onto the immobilized neutravidin. Figure 1 is a response curve for this immobilization technique. An alternative method that physisorbs the neutravidin directly to the gold is also described.

Materials and Methods

All reagents were obtained from Sigma-Aldrich unless otherwise stated. Note: Always use gloves when handling reagents and sensors. Neutravidin and biotinylated BSA were obtained from Pierce. Ligands were biotinylated using biotin-$(PEO)_4$-NHS from Pierce according to the manufacturers method. This reagent yielded superior results compared to other biotinylation reagents. Ensure adequate safety precautions are taken when handling all reagents. Refer to relevant MSDS for guidelines.

Immobilization of Ligand via Neutravidin-biotin Affinity Capture

Cleaning Gold

Use an ethanol saturated kimwip to gently wipe the gold surface thus removing contaminants. Be careful to ensure that no contact is made with the plastic surrounding the gold surface of Spreeta.

Neutravidin-biotin Method 1

1. Dock sensor with fluidics system, initialize, normalize and equilibrate in running buffer (e.g. PBS, pH 7.4).
2. Inject 100µl of biotinylated BSA (PIERCE) (1 mg/ml in PBS, pH 7.4) at 20µl/min. Expect binding response of 1000 -1500 µRIU. Remove excess protein by injecting 15 mM HCL for 30 sec.
3. Inject 100 µl of neutravidin at 20 µg/ml in PBS, pH 7.4. Expect binding response of 2000 to 2500 µRIU.

4. Dissolve biotinylated-ligand at 1 to 10 µg/ml in PBS, pH 7.4, and inject at 20 µl./min for 1 to 10 min. Expect a response of 1000 to 2000 µRIU for biotinylated antibody.
5. Check non-specific binding (NSB) by injecting 100 µl of BSA, and/or Ovalbumin, at 1 mg/ml in PBS, pH 7.4, for 5 min. This also doubles as a blocking step.

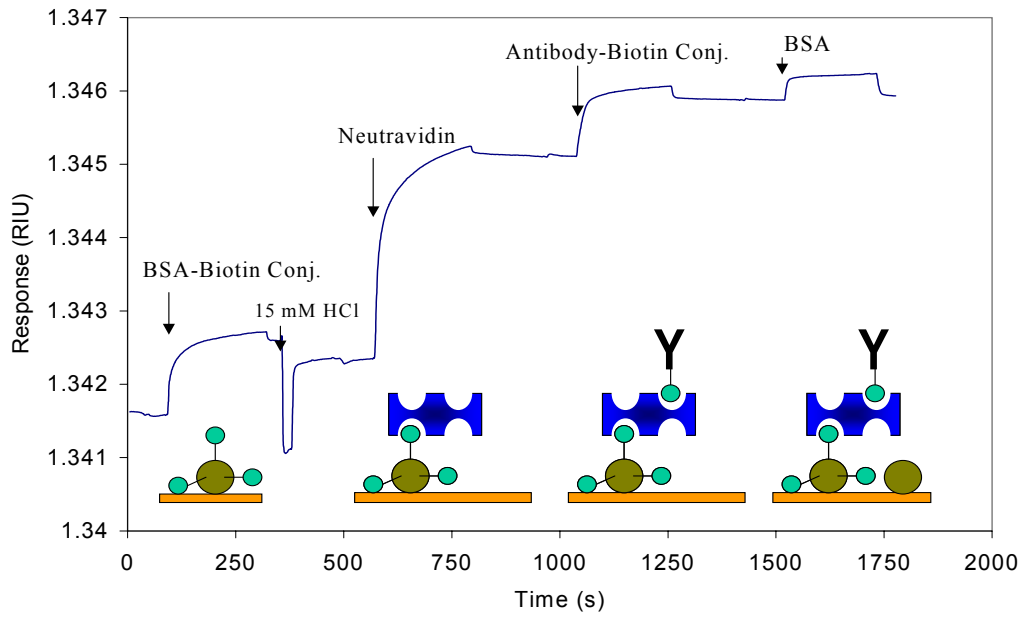

Figure 1. Response curve for immobilization of biotinylated Ligand.

Neutravidin-biotin Method 2

1. Dock sensor with fluidics system, initialize, normalize and equilibrate in running buffer (e.g. PBS, pH 7.4).
3. Inject 100 µl of neutravidin at 100 µg/ml in PBS, pH 7.4.
4. Dissolve biotinylated-ligand at 1 to 10 µg/ml in PBS, pH 7.4, and inject at 20 µl./min for 1 to 10 min.
5. Optional: Check non-specific binding (NSB) by injecting 100 µl of BSA, and/or Ovalbumin, at 1 mg/ml in PBS, pH 7.4, for 5 min. This also doubles as a blocking step.
6. Optional: Check binding activity of immobilized ligand by injecting antigen.

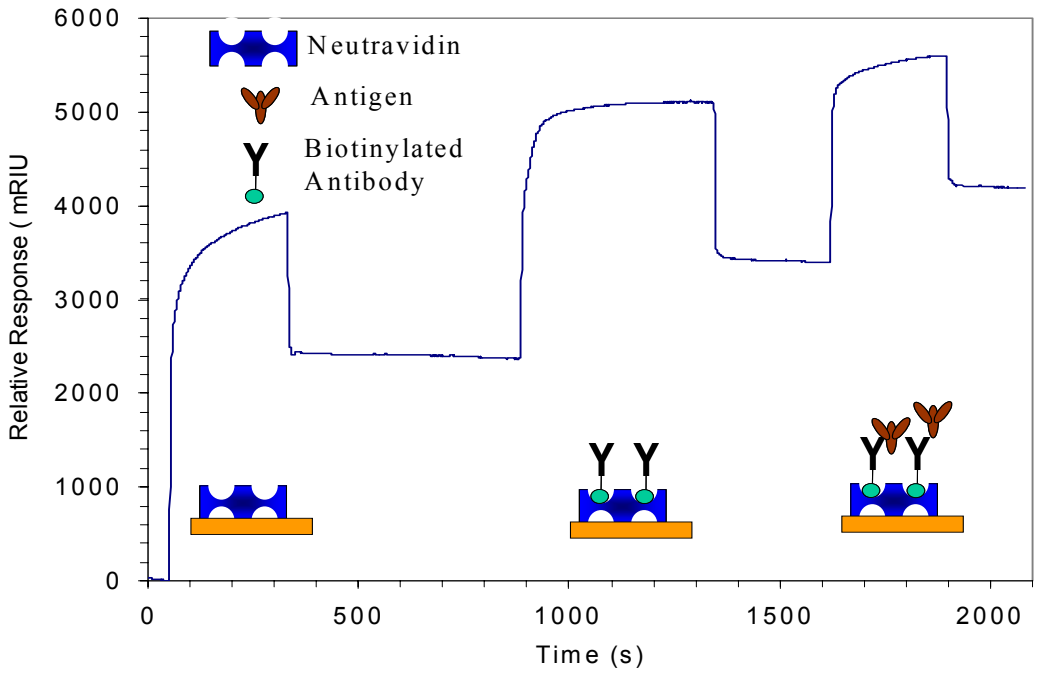

Figure 2. Direct immobilization of neutravidin (2400 μRIU) to gold surface followed by affinity capture of biotinylated antibody (1000 μRIU). The third injection is antigen (800 μRIU), and is used to confirm the binding activity of the immobilized antibody.

Materials and Methods

All reagents were obtained from Sigma-Aldrich unless otherwise stated. Note: Always use gloves when handling reagents and sensors. N-Succinimidyl 3-[2-pyridyldithio]propionate, BSA-biotin and neutravidin were obtained from Pierce. Ensure adequate safety precautions are taken when handling all reagents. Refer to relevant MSDS for guidelines.

Immobilization of Ligand via Neutravidin-biotin Affinity Capture

Cleaning Gold

Use an ethanol saturated kimwip to gently wipe the gold surface thus removing contaminants. Be careful to ensure that no contact is made with the plastic surrounding the gold surface of Spreeta.

Antibody Reduction

1. Weigh out 1 g of glyoxal agarose. Add 15 mls of 0.1 M NaOH then add 0.15 g Dithio-DL-threitol (DTT). Incubate overnight at RT.
2. Add 0.5 mls of the 1,4, DTT-agarose to a column and equilibrate with 0.1 M phosphate buffer, pH 8.0.
3. Activate the column by adding 1 ml of 10 mM DTT in 0.1 M phosphate buffer, pH 8.0, containing 1 mM EDTA.
4. Wash column with 20 column volumes of 0.1 M phosphate buffer, pH 8.0.
5. Add the antibody to be reduced in 0.1 M phosphate buffer, pH 8.0, containing 1 mM EDTA. Recover fractions and collect all samples. Read the absorbance at 280 nm to determine which fractions contain the eluted antibody. Pool these fractions, aliquot and freeze at -20°C until required.

Ligand-Thiol Coupling Method

1. Prepare 1 ml BSA-biotin (1 mg/ml) in PBS.
2. Place 10 μl of BSA-biotin onto the cleaned gold surface and incubate for 15 min at room temperature.
3. Rinse with 30 mM HCl and then with excess water.
4. Dock sensor with fluidics, initialize, normalize and equilibrate in running buffer (i.e. PBS, pH 7.4)
5. Inject 100 μl of neutravidin (100 μg/ml) in PBS, pH 7.4, for 5 min.
6. Activation: Prepare 1mg/ml N-Succinimidyl 3-[2-pyridyldithio]propionate (SPDP) in PBS, pH 7.4. Inject 200 μl at 20 μl/min.
7. Inject reduced antibody (100 μg/ml in 10 mM acetate, pH 4.2), for 5-10 min. A 1 min injection of 20 mM HCl is injected to remove weakly adsorbed protein (see Figure 1).
8. Capping: Inject mercaptoethanol (1 mM in 0.1 M acetate buffer, pH 4.2, containing 1 M NaCl) for 5 min. The mercaptoethanol removes any remaining pyridyl groups leaving a hydrophilic surface. The surface is now ready for use.

Regeneration

After the surface is exhausted it is possible to regenerate the surface as follows.

1. Regeneration: Inject 100 mM DTT in 0.1 phosphate buffer, pH 8.5.
2. Inject 10 mM pyridyl disulfide in 20%ethanol in 0.1 M phosphate buffer, pH 8.0, for 5 min.
3. Continue from step 7 above.

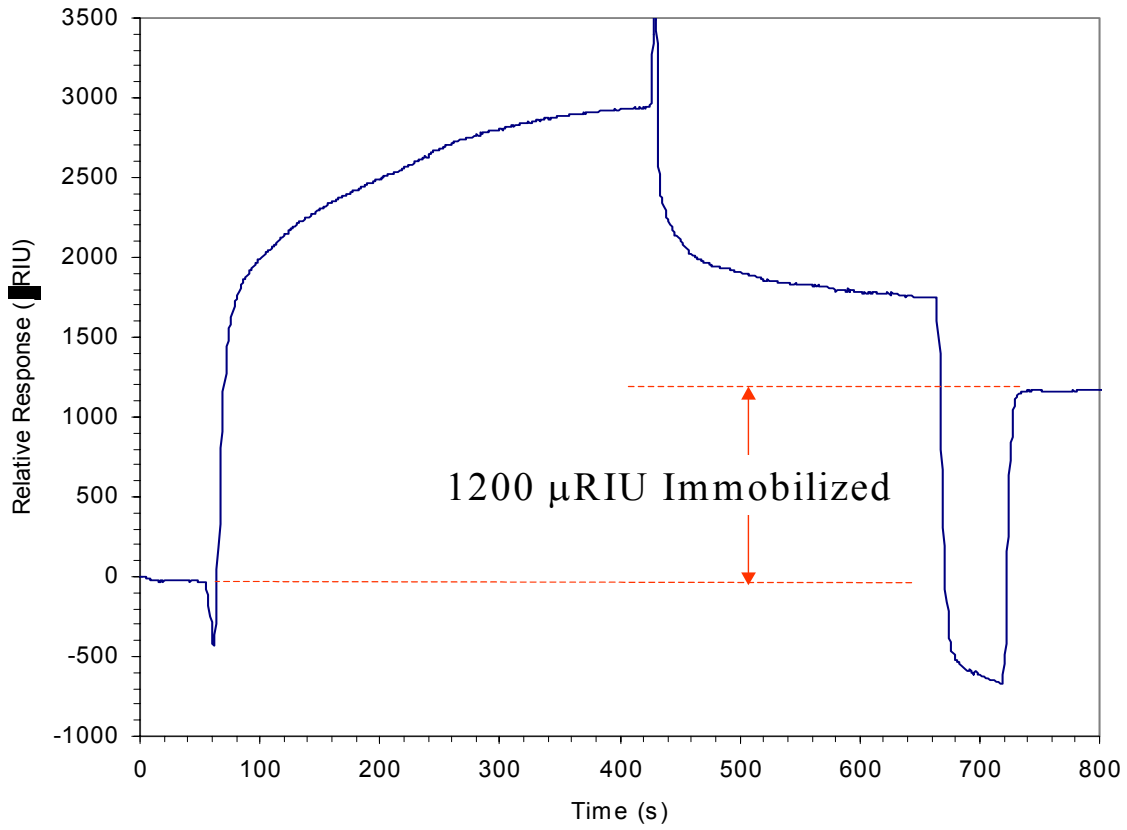

Figure 1. Injection of reduced goat IgG over an SPDP treated protein coated surface. The reduced antibody is linked to the surface by displacing pyridyl groups via disulfide exchange. An acid wash removed weakly adsorbed material leaving 1200 µRIU of antibody immobilized.