



APPLICATION BRIEF #003

Spreeta™

Ligand Immobilization of
Ligand: Method 3
Disulfide Exchange

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Immobilization of Ligand: Method 3

ABSTRACT

In this method a thiolated ligand is linked to a gold surface that is pre-coated with pyridyl disulfide groups. The ligand is covalently linked to the surface by disulfide exchange. It is possible to regenerate this surface using disulfide reductants.

Introduction

Immobilization of the ligand via a free thiol group allows more directed immobilization as there are usually only a few available per molecule. Many proteins lack available thiols and thiols are either chemically substituted or available disulfides are reduced. This attachment chemistry can be performed on the cross-linked protein films outlined in Notes 1 & 2.

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 kimwipe 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.

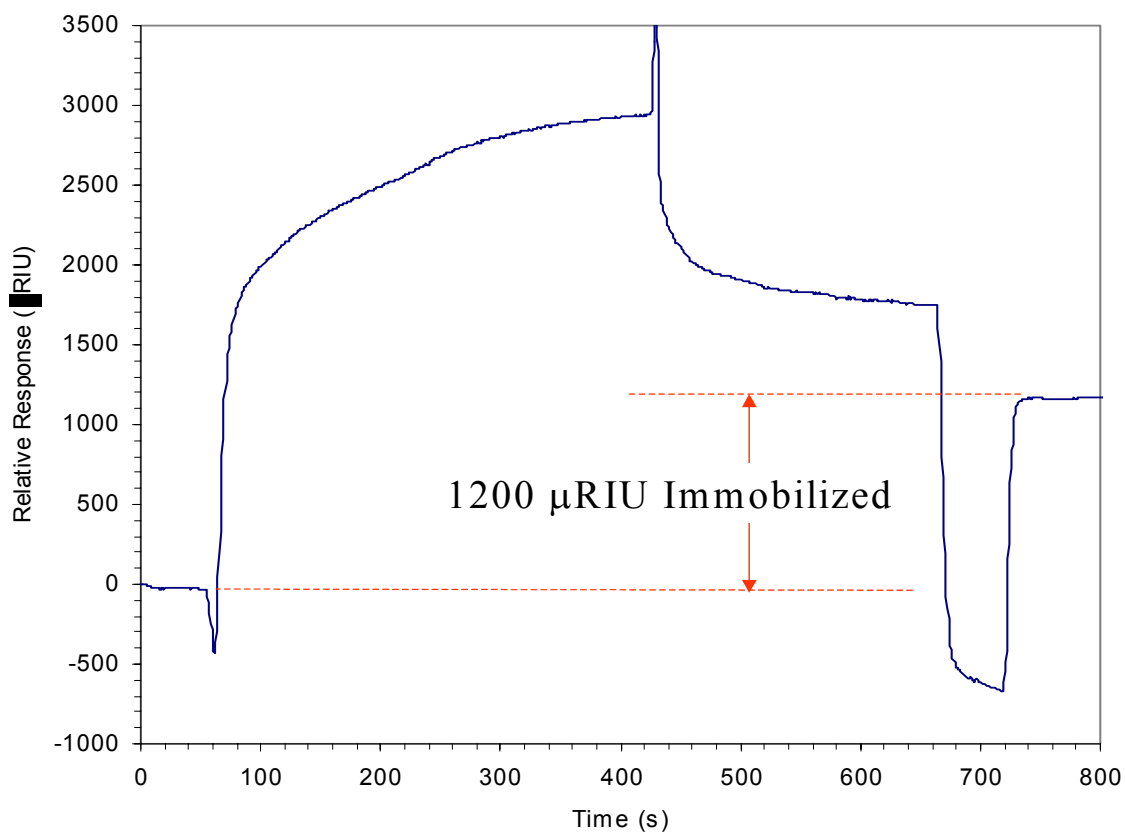


Figure 1. Injection of reduced goat IgG over an SPDP treated protein coated surface. The reduced antibody is linked to the surface by displacing pyridyl groups via disulfide exchange. An acid wash removed weakly adsorbed material leaving 1200 μ RIU of antibody immobilized.