

SpreetaTM

Immobilization of Ligand: Method 2 (Avidin-biotin Ligand Immobilization)

Application Brief

Number 002

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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 & Methods

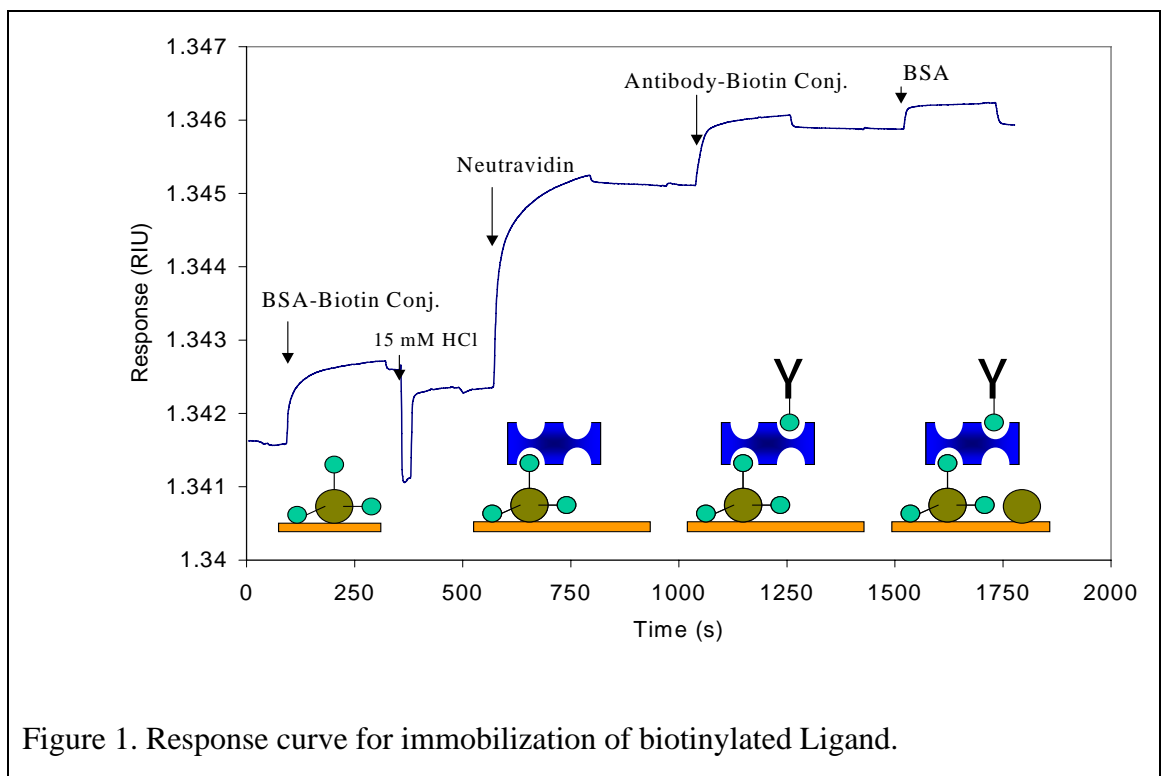
All reagents were obtained from Sigma-Aldrich unless otherwise stated. Note: Always use gloves when handling reagents and sensors. Neutravidin and biotinylated BSA were obtained from Pierce. Ligands were biotinylated using biotin-(PEO)₄-NHS from Pierce according to the manufacturers method. This reagent 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 100 μ l of biotinylated BSA (PIERCE) (1 mg/ml in PBS, pH 7.4) at 20 μ l/min. Expect binding response of 1000 -1500 μ RIU. Remove excess protein by injecting 15 mM HCL for 30 sec.
3. Inject 100 μ l of neutravidin at 20 μ g/ml in PBS, pH 7.4. Expect binding response of 2000 to 2500 μ RIU.
4. Dissolve biotinylated-ligand at 1 to 10 μ g/ml in PBS, pH 7.4, and inject at 20 μ l./min for 1 to 10 min. Expect a response of 1000 to 2000 μ RIU for biotinylated antibody.
5. Check non-specific binding (NSB) by injecting 100 μ l of BSA, and/or Ovalbumin, at 1 mg/ml in PBS, pH 7.4, for 5 min. This also doubles as a blocking step.



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 $\mu\text{g}/\text{ml}$ in PBS, pH 7.4.
4. Dissolve biotinylated-ligand at 1 to 10 $\mu\text{g}/\text{ml}$ in PBS, pH 7.4, and inject at 20 $\mu\text{l}/\text{min}$ 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.

