TEXAS INSTRUMENTS

Spreeta™

The Binding of Neutravidin Followed by the Attachment of Biotinylated Antibodies to the Spreeta Surface

Application Brief

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The Binding of Neutravidin Followed by the Attachment of Biotinylated Antibodies to the Spreeta™ Surface

ABSTRACT

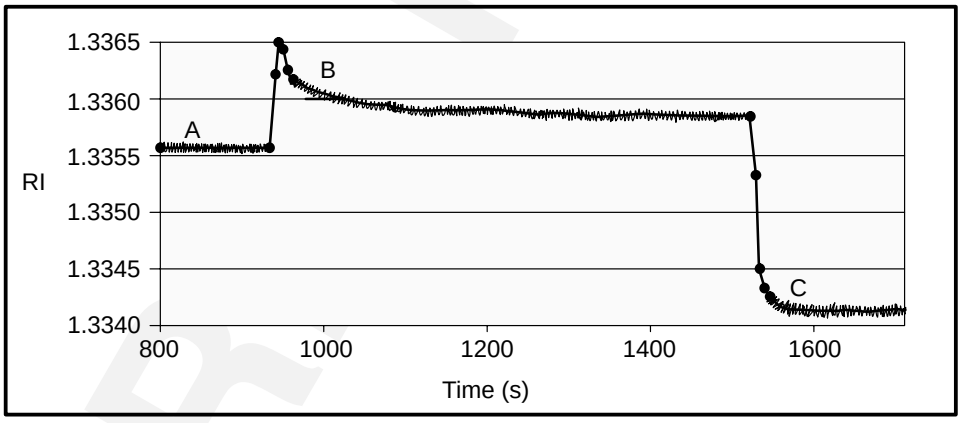
This process describes the binding of neutravidin to a clean gold surface followed by the attachment of functionally active biotinylated antibodies to the surface of the Texas Instruments (TI™) Spreeta sensor, which is included in the *Spreeta Evaluation Kit* (formerly known as the *TISPR-1 Experimenter's Kit*†).

Introduction

There are many different ways to attach antibodies (and other bio-recognition elements) to the surface of a Spreeta sensor. This application brief provides one protocol for use with biotinylated antibodies. As with all bio-attachment procedures, this one may not be suitable for your particular application; further optimization may be necessary.

Surface Cleaning

Before attaching any bio-recognition elements to the surface of the Spreeeta sensor, a gold surface cleaning procedure should be performed (see TI Application Brief 002, *Gold Spreeta™ Sensor Surface Cleaning*). For example, rinse the surface with phosphate buffered saline (PBS) for a few minutes and then switch over to a NaOH/Triton X-100 solution for approximately two minutes. Then, switch back to PBS and establish a good baseline (<1e–5/min), as shown in Figure 1.



NOTES: 1. A = The baseline with PBS running buffer
B = The application of 0.12 N NaOH in 1% Triton X-100
C = The new baseline with PBS running buffer on the cleaned gold surface
2. The refractive index shift between A and C (approximately 1.5×10^{-3}) is typical, but will depend on how dirty the gold surface was prior to cleaning.

Figure 1. In-Situ Cleaning of the Spreeta Gold Surface

† This test kit is being sold by Texas Instruments (TI) for experimental purposes only and not for commercial use.
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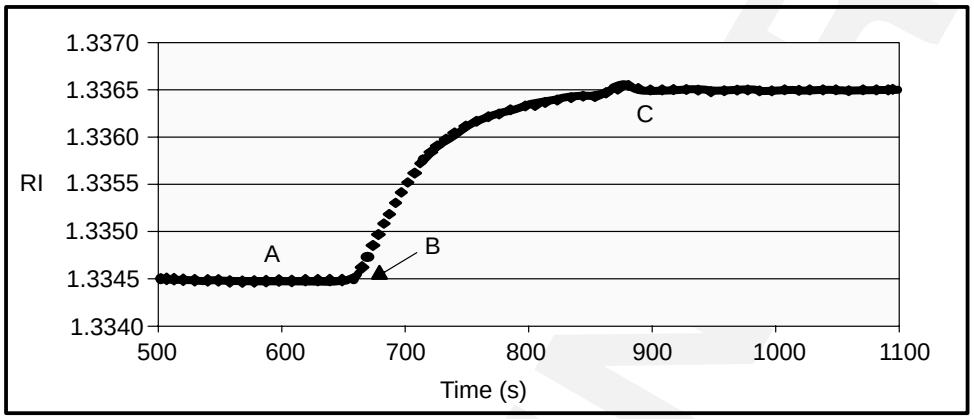
Surface Avidination

When the gold surface has been cleaned, and a steady PBS baseline has been established, begin flowing a 50-ug/ml solution of neutravidin in PBS. You should then see a normal protein binding curve. A total refractive index increase of between 1 and 2×10^{-3} should occur within five minutes. Revert to PBS, and less than 5% of the bound neutravidin should rinse off, as shown in Figure 2.



NOTES: 1. A = The baseline with PBS running buffer
B = The application of 20 ug/ml neutravidin in PBS
C = The new baseline with PBS running buffer on the cleaned gold surface
2. The refractive index shift between A and C (approximately 2×10^{-3}) is typical, but depends on how clean the gold surface was prior to avidination.

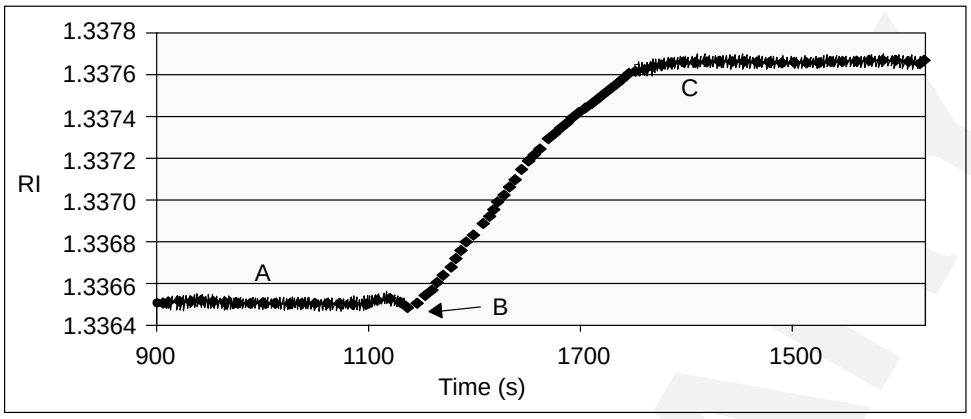
Figure 2. Binding of Neutravidin to a Clean Spreeta Gold Surface

Biotinylated Antibody Attachment

Antibodies are often available for purchase in a biotinylated form; but, they can also be biotinylated using a simple procedure that minimizes the degree of biotinylation and reduces the likelihood of modifying the antigen binding site. Such a procedure was used to produce biotinylated anti-dinitrophenol (DNP) antibodies for this work[1].

To attach an anti-DNP antibody, apply approximately 3 ml of a 3 ug/ml solution of biotinylated anti-DNP antibody in PBS to the avidinated Spreeta surface at a flow rate of approximately 0.3 ml/min.

This is sufficient to saturate the avidinated surface, as seen from the protein binding curve shown in Figure 3. A refractive index increase of approximately 1×10^{-3} is seen and very little of the antibody is rinsed off after you have switched back to the normal PBS running buffer. These values are typical.

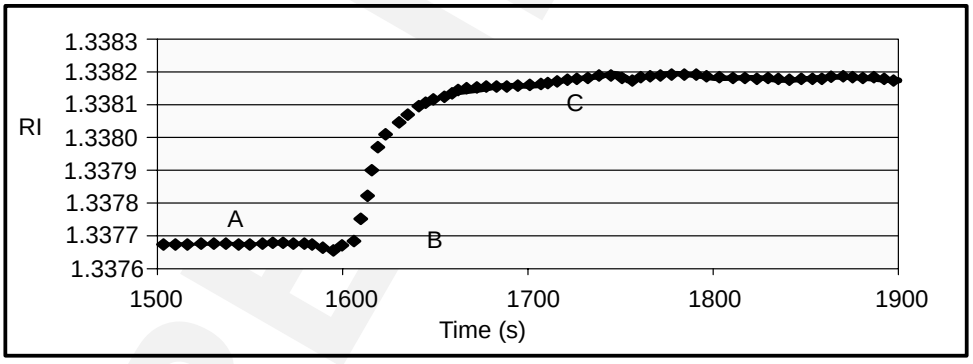


NOTES: 1. A = The baseline with PBS running across a neutravidinated surface
B = The application of 3 ug/ml biotinylated anti-DNP antibodies in PBS
C = The new baseline with PBS running buffer
2. The refractive index shift between A and C (approximately 1.2×10^{-3}) is typical.

Figure 3. Binding of Biotinylated Anti-DNP Antibodies to a Neutravidinated Spreeta Gold Surface

Evaluation of Antibody Activity

Following antibody attachment, a PBS/Triton X-100 baseline should be established. Triton X-100 is used to help reduce non-specific binding (NSB) antigen. In this case, 10 ug/ml DNP-bovine serum albumin (BSA) in PBS/0.1% Triton X-100 is applied to the surface. The resulting antigen-binding curve is obtained with a refractive index increase of approximately 5×10^{-4} , as shown in Figure 4.



NOTES: 1. A = The baseline with PBS + 0.1% Triton X-100 running across surface coated with biotinylated anti-DNP antibodies
B = The application of 3 ug/ml DNP-BSA, also in PBS + 0.1% Triton X-100
C = The new baseline with PBS + 0.1% Triton X-100 running buffer
2. The refractive index shift between A and C (approximately 5×10^{-4}) is typical.

Figure 4. Binding of DNP-BSA to Biotinylated Anti-DNP on a Spreeta Gold Surface

Controls

Blocking agents such as BSA can be used following surface avidination as well as after antibody attachment. Also, antibody attachment can be performed in the presence of 0.1% Triton X-100. Specificity was generally checked at appropriate times using BSA and other irrelevant proteins, and NSB was always less than 3% of specific binding for this system.

Summary

The procedure described here demonstrates one method for attaching functionally active antibodies to the Spreeta surface. This procedure may be modified to incorporate any one of a vast assortment of antibodies, although levels of activity will vary. More information on Spreeta is available at www.ti.com/spreeta. Send all questions and comments to spreeta@ti.com.

References

1. Wolf, C. and D.S. Hage, “Studies on the rate and control of antibody oxidation by periodate” *Analytical Biochemistry*, **231**, 123–130, 1995.
2. *Spreeta Evaluation Kit User’s Guide*, Texas Instruments Incorporated, Dallas, September 1999.