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Reversible Capture of Biotinylated Molecules for Biacore™ Analysis Using Biotin CAPture Kit

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Introduction

Label-free interaction analysis provides key binding data related to molecular function, such as specificity, affinity and kinetics. In such studies, biotinylated molecules are commonly investigated by direct capture on a streptavidin-derivatized sensor surface. This is rapid and requires only small amounts of biotinylated molecules, but these surfaces have the disadvantage that the biotinylated molecules cannot be removed.

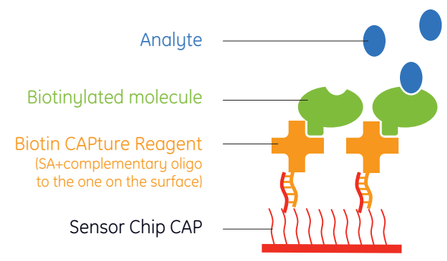
Here we describe a new product that enables reversible biotin capture. Streptavidin conjugated with an oligonucleotide is stably hybridized to a complementary sequence immobilized on a new sensor chip, Sensor Chip CAP. This has low unspecific binding comparable to other Biacore sensor chips.

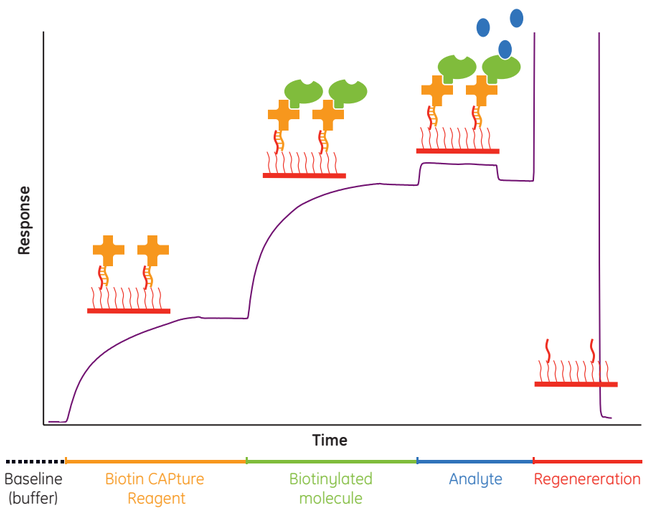
This results in a biotin capturing surface, that can be completely regenerated and reused repeatedly.

A New Capture Concept

Setting Up a Biotin CAPture Run

1. Biotin CAPture Reagent is hybridized to the surface.
2. Biotinylated molecule is captured to the Biotin CAPture Reagent.
3. Analyte interaction with capture molecule is studied.
4. The surface is regenerated and rebuilt with new Biotin CAPture Reagent in the next cycle.





When to Use Biotin CAPture Kit

The kit is the ideal choice when:

- there is little time or prior knowledge available to develop immobilization and regeneration conditions, for any ligand
- regeneration of the biotinylated molecule is difficult or the conditions are unknown
- working with unstable ligands that are sensitive to immobilization at low pH
- easy changes of captured interactant are desired
- the user wants to minimize chip costs when performing only short assay runs

The kit can be used for all common Biacore applications.

Study of the Interaction of TNF-α with Fab Fragments

(In Collaboration with Dr David Myszka, University of Utah, USA)

The challenge in studying TNF-α is that it is a non-covalent trimer, which falls apart if one tries to regenerate the surface. This makes work with standard immobilization difficult. By using Biotin CAPture Kit it was possible avoid regeneration.

The results demonstrate that Biotin CAPture Kit allows for ranking of Fab fragment binding to TNF-α.

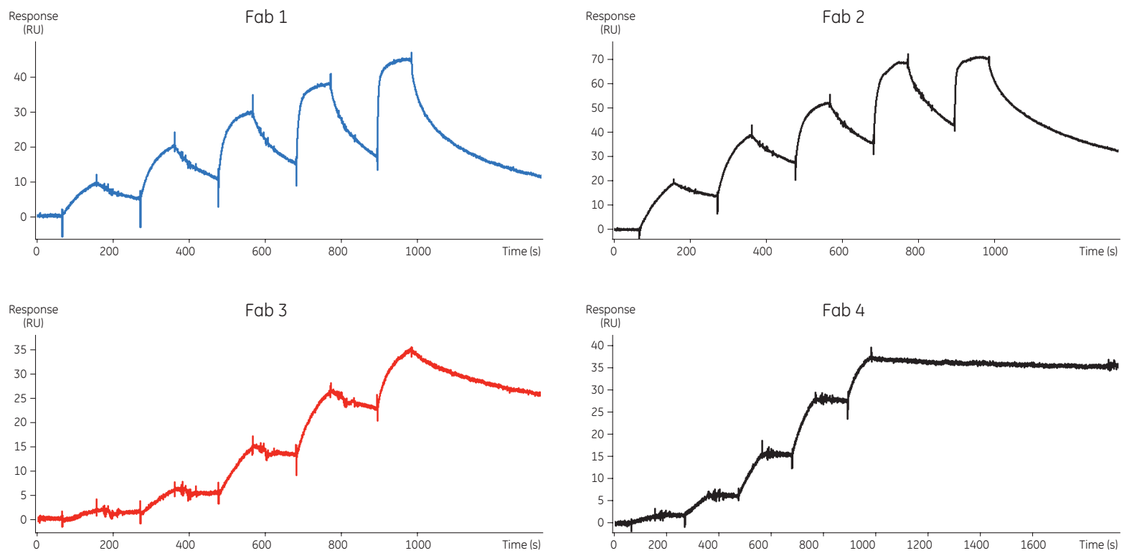


Fig 2. Single Cycle Kinetic runs, here showing ranking of four different Fabs in the order of decreasing dissociation rates.

Interaction of Low Molecular Weight Compounds with Kinase p38

(In Collaboration with Dr David Myszka, University of Utah, USA)

Kinases are unstable proteins that are sensitive to the low pH required for amine coupling as well as during regeneration. The use of Biotin CAPture Kit allows these problems to be avoided since biotinylation is carried out at neutral pH and regeneration is not needed. The figures below show overlays of replicate injections of five compounds at six different concentrations, fit to a 1:1 interaction model.

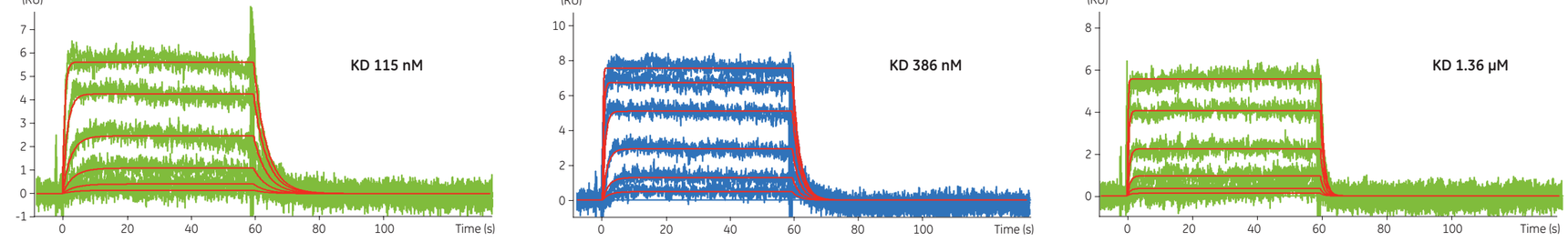


Fig 3. Fitted Multi Cycle Kinetics for five different lmw substances.

By using Biotin CAPture Kit it was possible to determine the kinetics for a number of compounds interacting with kinase p38, a ligand that does not tolerate amine coupling at low pH and harsh regeneration conditions.

Biotin CAPture Kit – Repeatability

When the capture surface is rebuilt in every cycle it is very important that the conjugate reaches the same level every time, i.e that the surface has the same properties during the entire run.

The variance for the Biotin CAPture Reagent level is <1% for 10 cycles, and <2% for 100 cycles.

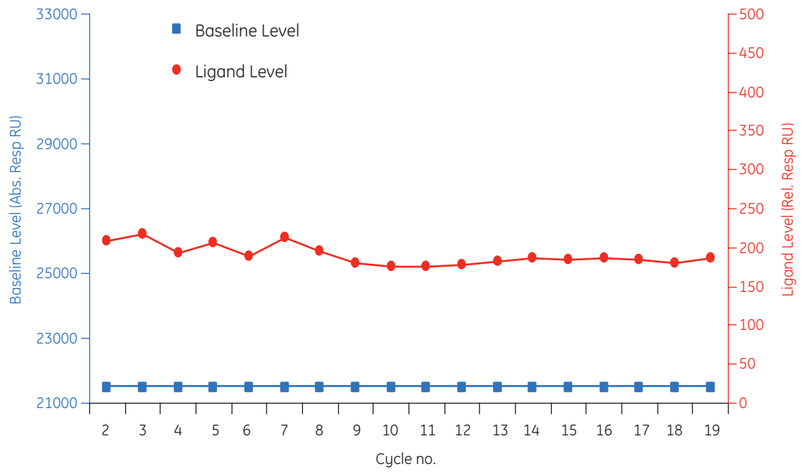


Fig 1. Baseline and ligand level from an epitope mapping assay. Courtesy of Dr Michael Schräml, Roche Diagnostics GmbH, Germany.

Kit Information

Sensor Chip CAP is built on a carboxymethylated dextran matrix to which a ss-DNA molecule is pre-immobilized. The Biotin CAPture Reagent is streptavidin, conjugated with the complementary ss-DNA molecule. The kit also contains a two-component regeneration solution which is mixed prior to use.

The kit is primarily for Biacore X100, T200, T100 and 2000/3000. Due to the demand for larger volumes the kit is not ideal for Biacore 4000 and A100, but is possible to run.

Kit reagents can be split into different runs and Sensor Chip CAP can be undocked and stored for future use.

Ordering information

Biotin CAPture Kit, Code no. 28-9202-33

Biotin CAPture Kit, Series S, Code no. 28-9202-34

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