

# Biotin CAPture Kit

Biotin CAPture Kit (Fig 1) is designed for the reversible capture of biotinylated molecules using a general, ligand-independent regeneration protocol. This offers several advantages such as (i) removing the need to establish regeneration conditions for a particular ligand; (ii) enabling you to work with unstable ligands; and (iii) eliminating the previous requirement to change sensor chips when you change ligands from one experiment to the other.

Direct immobilization of a ligand to a sensor chip surface via covalent bonds is the most widely used immobilization technique in biomolecular interaction studies. Although this results in a very stable ligand surface, the need to first scout for, and then optimize immobilization conditions can be tedious and costly with regards to the use of sensor chips and coupling reagents. Use of Biotin CAPture Kit avoids the efforts associated with the development of direct immobilization via covalent bonds.

The biotin-streptavidin method is an alternative to direct immobilization of a ligand on a sensor chip through covalent bonds. Normally, the biotinylated ligand is first immobilized onto a streptavidin (SA) surface followed by the injection of the analyte sample so that the interaction between ligand and analyte can be measured. Finally, the ligand is regenerated in preparation for a new sample cycle. This means that a regeneration protocol has to be developed for each ligand. In addition, you will have to prepare a new sensor chip if you want to study a new ligand. This process can be arduous and costly. In certain cases where the ligand is unstable or a regeneration condition cannot be established, the proposed experiment would have to be abandoned. Application of Biotin CAPture kit overcomes the limitations of the biotin-streptavidin immobilization method.



**Fig 1.** Biotin CAPture Kit is designed for reversible capture of biotinylated molecules for subsequent interaction analysis in Biacore systems.

## **Biotin CAPture Kit delivers:**

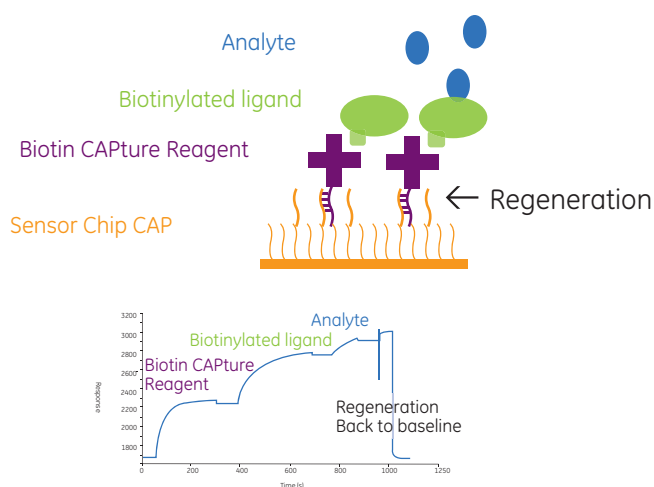
- **Speed and convenience:** Study interactions without the need to know or establish immobilization methods or regeneration protocols. Only biotinylation of the ligand is required.
- **Extended experimental range:** Investigate unstable ligands or ligands for which no regeneration can be established. This is possible since the sensor chip surface is regenerated and rebuilt in each cycle using a standardized protocol.
- **Affordability:** Save cost, time, and effort by retaining the same sensor chip when you need to change ligands between experiments.



## Method overview

### Immobilization techniques

The kit is based on a novel capture concept that allows for the reversible capture of biotinylated molecules and generic regeneration (Fig 2). Sensor Chip CAP is built on a carboxymethylated dextran matrix to which an ss-DNA molecule is pre-immobilized. Biotin CAPture Reagent consists of streptavidin conjugated with the complementary ss-DNA molecule. The coupling process is as follows: (i) Biotin CAPture Reagent is injected and hybridized to the surface of the sensor chip; (ii) the biotinylated ligand is then injected and captured; (iii) then the analyte sample is injected. Finally, the sensor chip surface is regenerated for a new experimental cycle.



**Fig 2.** Schematic representation of the novel capture concept used in Biotin CAPture Kit.

## Contents of Biotin CAPture Kit

The kit is available in two versions—one for each sensor chip format. The contents of the kit are summarized below.

**Table 1.** Biotin CAPture Kit contents

Biotin CAPture Kit <sup>1</sup>	Biotin CAPture Kit, Series S <sup>2</sup>
Sensor Chip CAP	Series S Sensor Chip CAP
Biotin CAPture Reagent	Biotin CAPture Reagent
Regeneration Stock 1	Regeneration Stock 1
Regeneration Stock 2	Regeneration Stock 2

<sup>1</sup>For use in Biacore X100 and Biacore 3000 (and other classic systems, although not verified for these).

<sup>2</sup>For use in Biacore T100. Although Biotin CAPture Kit can also be used in Biacore A100 system, factors such as chip utilization, flow rate, and the cycle times required by the kit may limit its use in this system.

## Range of Biotin CAPture Kit

*The capacities of the kits in Biacore systems are as follows:*

### Biotin CAPture Kit

- Biacore X100: 80 injections
- Biacore 3000: 140 injections

### Biotin CAPture Kit, Series S

- Biacore T100: 100 injections

Differences in kit range between systems are caused by different sample over-head volumes or fluidic dead volumes. The higher flow rate in Biacore A100 system limits the capacity of the kit to only 20 injections in this system.

### Binding capacity

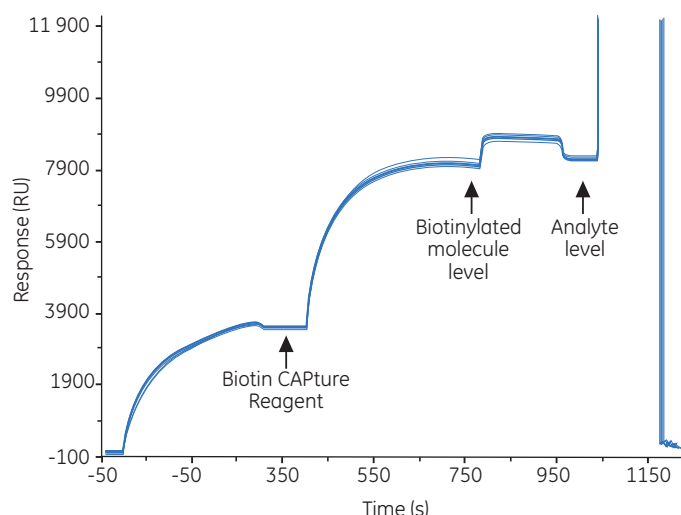
The binding capacity of the biotin-binding surface varies according to the size of the biotinylated ligand in the same way as it does for Sensor Chip SA but it is typically in the range of 1500 to 3000 RU for a ligand of molecular weight 150 000. This is lower than that for Sensor Chip SA, but it is sufficient for most of the applications and analyses performed in Biacore systems. The relatively low binding capacity may be a limiting factor in applications with low molecular weight analytes (e.g., < 5000).

### Samples

The kit is compatible with most of the samples and analytes used in Biacore systems. However, it is not suitable for experiments involving DNA-binding proteins, oligonucleotides or enzymes that degrade DNA because the assay includes oligonucleotides. In addition, serum or plasma samples tend to produce high levels of nonspecific binding to the sensor chip surface. Addition of low molecular weight dsDNA to the sample can sometimes prevent this type of nonspecific binding.

### Reproducibility

To ensure that the sensor chip surface retains the same properties throughout a run, it is important that each regeneration cycle produces the same response with Biotin CAPture Reagent. The binding level was highly reproducible at each stage of the assay as shown in Figure 3 and Table 2.



**Fig 3.** Overlay plot of 10 replicate cycles using Biotin CAPture Kit. Sample kindly provided by Affibody™, Sweden.

**Table 2.** Reproducibility of binding level responses using Biotin CAPture Kit

	10 cycles [% coefficient of variation (cv)]	100 cycles (% cv)
Biotin CAPture reagent level	0.8	1.1
Biotinylated molecule level	1.4	2.0
Analyte level	8.6	8.7

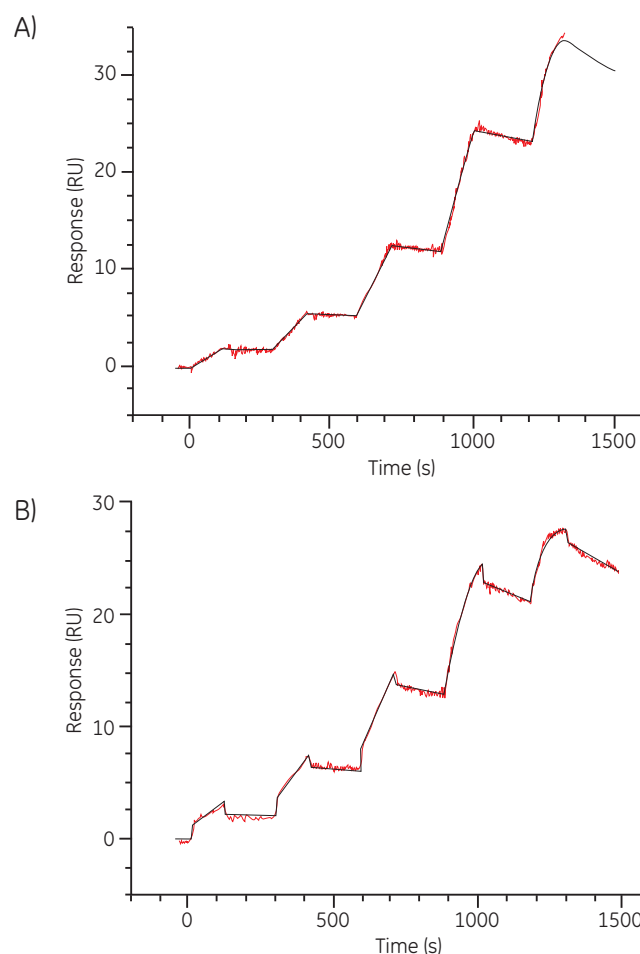
## Support for Biotin CAPture Kit in Biacore systems

- **Biacore X100:** Biacore X100 software (v2.0 and later) provides full support for Biotin CAPture Kit through a guided workflow in the base unit. It is capable of running both single- and multi-cycle kinetics.
- **Other Biacore systems:** Biotin CAPture Kit can be used in all other Biacore systems (except Biacore S51 and Biacore Flexchip) using MDL or Method Builder and the custom sensor chip option.

## Application of Biotin CAPture Kit

### Single-cycle kinetic analysis of the interaction between Affibody molecule Z477 and Human Epidermal Growth Factor Receptor 2 (HER2)

To study the protein-protein interaction between Z477 and HER2, we performed two single-cycle kinetics runs on a Biacore X100 system using Sensor Chip SA and Biotin CAPture Kit. The results in Figure 4 and Table 4 show that the performance of Biotin CAPture Kit in kinetic characterization is comparable to that of Sensor Chip SA.



**Fig 4.** Fitted single-cycle kinetics for (A) Biotin CAPture Kit and (B) Sensor Chip SA.

**Table 4.** Kinetic constants obtained with Biotin CAPture Kit and Sensor Chip SA, respectively

	$k_a$	$k_d$	$K_D$	$R_{max}$	$tc$
Biotin CAPture Kit	$5.9 \times 10^6$	0.0008	135 pM	34	$9.1 \times 10^6$
Sensor Chip SA	$2.0 \times 10^6$	0.0007	350 pM	27	$7.1 \times 10^6$

## Summary

Biotin CAPture Kit is an easy-to-use, versatile, and reliable immobilization tool for studying biomolecular interactions on Biacore systems. It overcomes the limitations of other immobilization methods and provides several advantages that allow you to investigate biomolecular interactions with speed and consistency. A comparative kinetic characterization produced similar results for both Biotin CAPture Kit and Sensor Chip SA.

## Ordering information

Product	Code no.
Biotin CAPture Kit	28-9202-33
Biotin CAPture Kit, Series S	28-9202-34

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