

# Biotin CAPture Kit

## Instructions for Use

### Product description

Product code:	28920233 (Biotin CAPture Kit) 28920234 (Biotin CAPture Kit, Series S)
Contents:	<ul style="list-style-type: none"><li>• One Sensor Chip CAP or one Series S Sensor Chip CAP</li><li>• Biotin CAPture Reagent, 50 µg/mL in HBS-EP buffer (0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005 Surfactant P20), 3.4 mL</li><li>• Regeneration Stock 1 (8 M guanidine hydrochloride), 16 mL</li><li>• Regeneration Stock 2 (1 M sodium hydroxide), 6 mL</li></ul>
Storage:	2°C to 8°C
Kit capacity:	<p>The kit provides sufficient volumes for 60 injections of Biotin CAPture Reagent and regeneration solution. The number of experimental cycles can be much greater depending on the instrument used and assay design, specifically the requirement to regenerate between cycles. Multi-needle instruments require each needle to have its own injection volume, which can result in lower total number of cycles compared to single-needle instruments.</p> <p><b>Note:</b> <i>Biotin CAPture Reagent (29423383) is available for separate purchase.</i></p> <p>For more details, visit <a href="https://www.cytiva.com/biacore">cytiva.com/biacore</a>.</p>
For use with:	Any Biacore™ system, except Biacore 4000 SPR system.

Safety: For use and handling of the product in a safe way, refer to the Safety Data Sheet.

**Note:** For research use only.

## Intended use

Biotin CAPture Kit enables reversible capture of biotinylated ligands for interaction analysis in Biacore systems. The ligand is captured on to Sensor Chip CAP via Biotin CAPture Reagent, which is a modified streptavidin. Sensor Chip CAP should only be used together with Biotin CAPture Reagent.

Regeneration of the surface after each analysis cycle removes Biotin CAPture Reagent as well as the ligand and any bound analyte. Fresh Biotin CAPture Reagent is attached to the surface for each cycle.

Sensor Chip CAP and Biotin CAPture Reagent contain deoxyribo-oligonucleotides. Therefore, the kit is not suitable for work with DNA-binding proteins or enzymes that degrade DNA.

The ligand capture capacity of Sensor Chip CAP and Biotin CAPture Reagent is typically about 1500 to 3000 RU for a ligand with  $M_r$  150 000.

Biotin CAPture Kit has been optimized for an assay temperature of 25°C.

## Preparations for use

### Ligand biotinylation

Substitution levels of one biotin residue per ligand molecule or less are recommended for capture using Biotin CAPture Kit. In general, procedures supplied with commercial biotinylation reagents tend to give higher substitution levels. When using N-hydroxysuccinimide (NHS)-biotin reagents for ligand biotinylation, reduce the concentration of reagent to less than 1 mole of biotinylation reagent per mole of ligand.

It is essential that excess biotinylation reagent is removed from the ligand preparation before capture, to avoid competition with the biotinylated ligand for binding to Biotin CAPture Reagent. Separate the biotinylated ligand from excess reagent using for example size-exclusion chromatography (micro-spin columns are recommended for volumes below 120  $\mu$ L to minimize dilution). Use two cycles of separation to ensure that no free reagent remains in the ligand preparation.

For updates on applications and scientific publications, refer to [cytiva.com/biacore](https://www.cytiva.com/biacore).

## Clean the flow system

Make sure that the flow system is clean before docking Sensor Chip CAP, particularly after experiments using other biotinylated molecules. Follow the steps below to perform a flow system cleaning.

Step	Action
1	Run the maintenance tool <b>Desorb</b> .
2	If the <b>Sanitize</b> maintenance tool has been run, prime all buffer inlets that will be used in the analysis with running buffer. Run running buffer in standby mode for at least 4 hours or overnight before docking Sensor Chip CAP.

**Note:**  
*Do not use plain water as running buffer. Water rinses sodium hypochlorite insufficiently compared to running buffer. Residues of sodium hypochlorite can damage sensor chips.*

## Rehydration of Sensor Chip CAP

Before first use, rehydrate the sensor chip to achieve full binding capacity and minimize response drift. If this is not done, the binding levels of Biotin CAPture Reagent will increase gradually during the first day of exposure to liquid.

To rehydrate the chip:

- Dock the chip in the instrument and leave on standby with running buffer or water at least overnight.

**OR**

- Follow the steps below:

Step	Action
1	Remove the sensor chip carefully from the plastic cassette. For details on chip handling, storage, and reuse, see <i>Biacore Sensor Surface Handbook</i> .
2	Place the sensor chip in a capped container containing distilled water at 55°C to 60°C. Allow the sensor chip to equilibrate to room temperature and leave to stand for at least 90 minutes.

**Note:**  
*Do not let the temperature exceed 70°C.*

**OR**

Place the sensor chip in a capped container containing distilled water or HBS-EP+ buffer at room temperature. Leave the sensor chip to stand overnight.

Step	Action
3	Dry the chip with oil-free compressed air or nitrogen.  <b>Note:</b> <i>Do not touch the gold chip.</i>
4	Place the chip in the cassette and dock it in the instrument.

## Regeneration solution

Regeneration solution is prepared from Regeneration Stock 1 and 2 provided in Biotin CAPture Kit. If regeneration using the standard procedure is not adequate (indicated by decreasing levels of captured ligand during the course of the assay), an additional regeneration is recommended. This can be necessary if the ligand has more than 2 biotin residues per molecule.

Follow the instructions below to prepare standard regeneration solution or additional regeneration solution.

Solution	Preparation
Standard regeneration	<p>Allow Regeneration Stock 1 to reach room temperature and make sure that any precipitate is dissolved.</p> <p>Mix the following:</p> <ul style="list-style-type: none"> <li>• 3 parts of Regeneration Stock 1 (8 M guanidine-HCl)</li> <li>• 1 part Regeneration Stock 2 (1 M NaOH)</li> </ul> <p><b>Note:</b> <i>Mixed regeneration solution should be used within 2 days.</i></p> <p><b>Note:</b> <i>Regeneration Stock 1 will precipitate on cold storage. However, the mixed regeneration solution can be kept cold for up to 2 days without risk of precipitation.</i></p>
Additional regeneration	<p>Prepare 30% acetonitrile in 0.25 M NaOH.</p> <p><b>Note:</b> <i>This solution is not included in Biotin CAPture Kit.</i></p>

# Recommended running conditions

## Running buffer

Biotin CAPture Kit is compatible with most running buffers used with Biacore systems. Choose running buffer according to the needs of the interaction to be studied.

## Reference surface

For applications that use a reference surface, prepare the surface with an injection of Biotin CAPture Reagent.

**Note:** *Do not use an unmodified surface as reference.*

## Surface conditioning

Condition the sensor chip surface with three one-minute injections of regeneration solution when the chip is docked for the first time or after storage. Conditioning is not necessary for a sensor chip that has been used and left in the instrument.

## Start-up cycles

One to three complete start-up cycles, including injections of Biotin CAPture Reagent, ligand, sample, and regeneration solution are recommended for newly docked chips to allow the surface to stabilize before analysis.

## Injection sequence

Each interaction analysis cycle using Biotin CAPture Kit is performed with the following basic sequence of injections:

1. Biotin CAPture Reagent

Biotin CAPture Reagent in the kit is ready to use. Do not dilute the reagent solution.

Recommended conditions: flow rate 2  $\mu\text{L}/\text{min}$  and contact time 5 min. Conditions can be set in the software for some Biacore systems. Response levels above 1500 RU are normal.

2. Ligand

Inject biotinylated ligand

Recommended conditions: flow rate and contact time for the injection will depend on the application requirements. Adjust the ligand concentration and contact time if necessary to control the amount of captured ligand.

### 3. Analyte

Recommended conditions: conditions will vary according to the requirements of the application. Serum or plasma samples tend to give fairly high levels of nonspecific binding to Sensor Chip CAP. Addition of degraded or low molecular weight double-stranded DNA (dsDNA) to the samples can sometimes counteract this non-specific binding. Good results have been obtained with dsDNA from salmon and herring at 1 mg/mL.

### 4. Regeneration

Use fresh regeneration solution (see [Regeneration solution, on page 4](#)).

Recommended conditions: flow rate 5 to 30  $\mu\text{L}/\text{min}$  and contact time 2 min. Regeneration solution has a high refractive index and will give a response that is off-scale in all Biacore systems. In some situations, an off-scale response can be shown as zero in the sensorgram.

If additional regeneration is needed, use a two-minute injection of 30% acetonitrile in 0.25 M NaOH (see [Regeneration solution, on page 4](#)).

Include an extra wash with running buffer after the last regeneration injection to wash the needle and flow system.

## Use of Sensor Chip CAP

It is recommended to use Sensor Chip CAP for up to 150 assay cycles per flow cell. Note that the maximum number of cycles can depend on the type of ligand.

For more information on running conditions for different applications, guides, lab protocols, and free eLearnings, visit [cytiva.com/biacore](http://cytiva.com/biacore).

## Storage and reuse of Sensor Chip CAP

For best performance, perform storage and reuse procedures described below consecutively with no waiting time in between.

### Storage

Sensor Chip CAP can be undocked and stored for up to 1 month after opening the package. Follow the steps below to undock and store the chip.

Step	Action
1	Undock the sensor chip and remove it from the instrument.
2	Remove the sensor chip support from the cassette with a pair of tweezers. Avoid touching the support with your fingers.

Step	Action
3	Place the support in a 50 mL tube containing buffer so that the support is completely covered. Cap the tube securely and store it in a refrigerator.
	<b>Note:</b> <i>Wet storage in HBS buffer at 2°C to 8°C is recommended.</i>

For further details on chip storage, refer to *Biacore Sensor Surface Handbook*.

## Reuse

Follow the steps below to reuse Sensor Chip CAP.

Step	Action
1	Take the support out of the tube using a pair of tweezers. Avoid touching it with your fingers.
2	Rinse the support with distilled water and shake it gently to remove most of the water.
3	Wipe the support and the glass side of the sensor chip dry with a lint-free tissue or dry with pressurized oil-free air or nitrogen. Do not wipe or dry the sensor surface itself. The glass sensor chip is fixed on the support so that the sensor surface is recessed with respect to the support.
4	Reinsert the support into the cassette with the glass side towards the label on the cassette. Do not force the support into the cassette. Insert the sensor chip in the instrument and dock it. Equilibrate the system with buffer by running the <b>Change solutions</b> or <b>Prime</b> command depending on the Biacore SPR system.

## Chemical resistance

The surface of Sensor Chip CAP is resistant to many buffers and solutions used in biochemical studies. For information about resistance to common agents compatible with Sensor Chip CAP, see the table below.

Agent	Concentration
Acetonitrile	30%
DMSO	10%
Ethanol	70%

<b>Agent</b>	<b>Concentration</b>
Ethanolamine	1 M
Ethylene glycol	100%
Glycine pH 1.5 to 3.0	100 mM
HCl	100 mM
NaOH	250 mM
NaCl	5 M
SDS	0.5%
Surfactant P20	5%
Urea	8 M

For information on resistance to other solutions, refer to a Cytiva representative.



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