

Biotin CAPture Kit

Instructions for Use

Product description

- Product codes: 28920233 (Biotin CAPture Kit)
28920234 (Biotin CAPture Kit, Series S)
- Kit contents:
- One Sensor Chip CAP or one Series S Sensor Chip CAP
 - 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
 - Regeneration stock 1 (8 M GuHCl), 16 mL
 - Regeneration stock 2 (1 M NaOH), 6 mL

Note:

Biotin CAPture Reagent (29423383) and Series S Sensor Chip CAP (29805820) are available for separate purchase.

For more details, visit [cytiva.com/biacore](https://www.cytiva.com/biacore).

Storage: +2°C to +8°C

Kit capacity:	<p>The kit provides sufficient volumes for 60 injections of Biotin CAPture Reagent and regeneration solution.</p> <p>The number of experimental cycles varies depending on the following:</p> <ul style="list-style-type: none"> • instrument used • assay design • need for regeneration between cycles <p>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>
For use with:	Any Biacore™ system.
Safety:	For use and handling of the product in a safe way, refer to the <i>Safety Data Sheet</i> .

Note: For research use only.

Intended use

Biotin CAPture Kit allows for 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.

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 unsuitable for work with DNA-binding proteins or DNA-degrading enzymes.

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 µL to minimize dilution). Use two cycles of separation to ensure that no free reagent remains in the ligand preparation.

For a Biacore-specific biotinylation protocol as well as 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. For cleaning, follow the step below.

Step	Action
1	Run the maintenance tool Desorb . OR Run the maintenance tool Desorb and sanitize . Note: <i>After using the Desorb and sanitize maintenance tool, run Change solutions or Prime with running buffer on all buffer inlets used during the analysis. Run running buffer in standby mode for at least four hours or overnight before docking the sensor chip. Do not use plain water as running buffer. Water does not rinse sodium hypochlorite as effectively as running buffer, and sodium hypochlorite residues damage sensor chips.</i>

Rehydrate Sensor Chip CAP

Before first use, rehydrate the sensor chip to achieve full binding capacity and minimize response drift. Otherwise, the binding levels of Biotin CAPture Reagent gradually increase during the first day of exposure to liquid.

To rehydrate the sensor chip, dock the sensor chip in the instrument and leave on standby with running buffer at least overnight. Or, to rehydrate the sensor chip outside of the instrument, follow the steps below:

Step	Action
1	Carefully remove the sensor chip from its plastic cassette. For details on sensor chip handling, storage, and reuse, refer to <i>Biacore Sensor Surface Handbook</i> available on cytiva.com/biacore .
2	Place the sensor chip in a capped container with deionized water at 55°C to 60°C. Leave the sensor chip submerged in the water for at least 90 minutes while it equilibrates to room temperature. Note: <i>Do not let the temperature exceed 70°C.</i> OR Place the sensor chip in a capped container with deionized water or HBS-EP+ buffer at room temperature. Leave the sensor chip to stand overnight.
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 sensor surface is recessed relative to the support, whereas the glass side is slightly raised. Note: <i>Do not touch the gold chip.</i>
4	Place the sensor chip in its cassette and dock the cassette into 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"> • 3 equal volumes of Regeneration stock 1 (8 M GuHCl) • 1 volume of Regeneration stock 2 (1 M NaOH) <p>Note: <i>Use the mixed regeneration solution within 2 days.</i></p> <p>Note: <i>Regeneration stock 1 precipitates on cold storage. However, the mixed regeneration solution can be stored cold without risk of precipitation.</i></p>
Additional regeneration	<p>Prepare 30% acetonitrile in 0.25 M NaOH.</p> <p>Note: <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 that are used with Biacore systems. Select a 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 1-minute injections of regeneration solution.

Note: *Conditioning the sensor chip surface is required when using a certain channel or flow cell for the first time or after storing the sensor chip. Conditioning is not required for a sensor chip that has been used and left on standby flow 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. Biotinylated ligand

Recommended conditions: flow rate and contact time for the injection 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 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

Sensor Chip CAP can be used for at least 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](https://www.cytiva.com/biacore).

Storage and reuse of Sensor Chip CAP

Storage

Sensor Chip CAP can be undocked and stored for up to 2 months after opening the package. To undock and store the chip, follow the steps below—without any delay between steps.

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.
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.
	Note: <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* available on [cytiva.com](https://www.cytiva.com).

Reuse

To reuse Sensor Chip CAP, follow the steps below—without any delay between steps.

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 deionized 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 relative 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 Change solutions or Prime 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%
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 agents, contact a Cytiva representative.

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