Biotin CAPture Kit

Instructions for Use

Product description

Order code:	28920233 (Biotin CAPture Kit) 28920234 (Biotin CAPture Kit, Series S)
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 Biotin CAPture Reagent can be ordered as a stand-alone product. Order code: 29423383 • Regeneration Stock 1 (8 M guanidine hydrochloride), 16 mL • Regeneration Stock 2 (1 M sodium hydroxide), 6 mL
Storage:	2°C to 8°C Regeneration Stock 1, should be stored at room temperature (precipitates at low temperature).
Kit capacity:	The contents of Biotin CAPture Kit are sufficient for 60 to 140 assay cycles in systems other than Biacore™ 4000 SPR system. For this system there are limitations in the use of the kit (see below). For more details, visit *cytiva.com/biacore*
Limitations of use:	Volumes of solutions supplied in Biotin CAPture Kit limit the usefulness of the kit with Biacore 4000 SPR system, and methodology support for using the kit with this system is not available.
Safety:	For use and handling of the product in a safe way, please 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 deoxyribooligonucleotides. The kit is primarily intended for protein-protein interaction studies. It 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.

Preparations for use

Ligand biotinylation

Substitution levels of about one biotin residue per ligand molecule 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 about 1 to 2 moles of 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.

Refer to *cytiva.com/biacore* for updates on applications and scientific publications

Cleaning 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.

Note: *Perform any cleaning steps before docking Sensor Chip CAP.*

Step	Action
1	Run the maintenance tool ***Desorb***.

Step	Action
2	If the ***Sanitize*** maintenance tool has been run, prime all buffer inlets that will be used in analysis with running buffer. ***Note:*** *The chip surface is sensitive to sodium hypochlorite residues.* ***Note:*** *Do not use plain water as running buffer at this stage.*

Rehydration of Sensor Chip CAP

To achieve full binding capacity and minimize response drift, the sensor chip should be rehydrated before first use. 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 re-use, see *Biacore Sensor Surface Handbook (BR100571)*.
2	Place the sensor chip in distilled water in a capped container at 55°C to 60°C then allow to stand at room temperature for at least 90 minutes. **OR** Place in distilled water or HBS-EP buffer at room temperature and allow to stand overnight. ***Note:*** *Do not let the temperature exceed 70°C.*
3	Dry the chip with oil-free compressed air or nitrogen. ***Note:*** *Do not touch the gold chip.*
4	Replace the chip in the cassette and dock it in the instrument.

Regeneration Solution

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

Solution	Preparation
Standard regeneration	Prepare regeneration solution by mixing 3 parts of Regeneration Stock 1 (8 M guanidine-HCl) with 1 part Regeneration Stock 2 (1 M NaOH). Use within 2 days. Warm the solution to room temperature and make sure any precipitate is dissolved before preparing regeneration solution. ***Note:*** *Regeneration Stock 1 will precipitate on cold storage. However, the mixed regeneration solution may be kept cold without risk of precipitation.*
Additional regeneration	If regeneration using the standard procedure is not adequate (indicated by decreasing levels of captured ligand during the course of the assay), a second regeneration injection of 30% acetonitrile in 0.25 M NaOH is recommended. This additional regeneration may be necessary in particular if the ligand has more than 2 biotin residues per molecule. ***Note:*** *This solution is not included in Biotin CAPture Kit.*

Run 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 being 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 assay performance to stabilize before analysis.

Injection sequence

Each interaction analysis cycle using the 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 µL/min, contact time 5 min. Conditions may be fixed in the software for some Biacore systems. Response levels of about 2500 to 5000 RU are normal.

2. Ligand

   Inject 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 µL/min, 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 may 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 the Sensor Chip CAP for maximum approximately 150 assay cycles per flowcell/spot. Note that the amount of cycles may depend on type of ligand.

Storage and re-use of Sensor Chip CAP

Sensor Chip CAP may be undocked and stored for up to 2 months after opening the package. Wet storage in HBS buffer at 2°C to 8°C is recommended.

Refer to *Biacore Sensor Surface Handbook (BR100571)* for details on chip storage.

Chemical resistance

The surface of Sensor Chip CAP is resistant to many buffers and solutions used in biochemical studies. See table below for information of common agents compatible with Sensor Chip CAP.

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

Refer to a Cytiva representative for information on resistance to other solutions.

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For local office contact information, visit cytiva.com/contact

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