

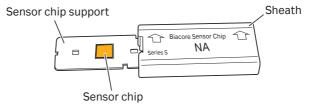
Series S Sensor Chip NA Instructions for Use

A Biacore Extend product

This product is part of the Biacore[™] Extend product line and is not a standard Biacore consumables product.

Product description

Product code:	29407997 (Package of one sensor chip)
	29699622 (package of three sensor chips)
Storage:	The use-before date applies to chips stored at 2°C to 8°C in unopened pouches.



The sensor chip is fixed to a polystyrene sensor chip support. Each cassette, consisting of a sensor chip and sheath assembly, is packed in a separate pouch and sealed under a nitrogen atmosphere.

Note: For research use only.

Application areas

Series S Sensor Chip NA is designed to capture biotinylated molecules to perform ligandanalyte interaction analysis primarily in low molecular weight applications.

For updates on applications and scientific publications, refer to cytiva.com/biacore, Biacore Consumables Selection Guide (CY14015), Biacore Sensor Surface Handbook (BR100571), and Biacore application guides.

Surface specificity

The surface consists of a carboxymethylated dextran matrix pre-immobilized with NeutrAvidin[™] and is ready for high-affinity capture of biotinylated ligands, such as peptides, proteins, and nucleic acids. Series S Sensor Chip NA provides a convenient alternative to covalent coupling for ligands that are difficult to immobilize directly or do not withstand covalent immobilization. Controlled biotinylation enables oriented capture.

Preparation of biotinylated ligand

Substitution levels of one biotin residue per ligand molecule or less are recommended for capture using Series S Sensor Chip NA. 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 the binding site on Series S Sensor Chip NA. Separate the biotinylated ligand from excess reagent using for example size-exclusion chromatography (micro-spin columns are recommended for volumes below 120 μ L to minimize dilution). Use two cycles of separation to make sure that no free reagent remains in the ligand preparation.

Immobilization overview

Biotinylated ligand is immobilized by non-covalent capture to NeutrAvidin.

An immobilization procedure of a biotinylated ligand on Series S Sensor Chip NA must include the following steps:

- conditioning of the sensor chip
- injection of biotinylated ligand
- wash after ligand injection

To create an immobilization procedure, refer to the Biacore software handbook of your instrument, and to the details in this instruction.

Preparations for immobilization

Preparing buffers and solutions

Select a running buffer compatible with your biotinylated ligand. If possible, include detergent in the running buffer used for immobilization. PBS-P+ or HBS-EP+ is recommended as running buffer. For more details on buffer selection, refer to the Biacore handbooks.

Note: All buffers should be filtered (0.22 μm). Premade buffers purchased from Cytiva do not need filtering.

Prepare the following solutions for immobilization on Series S Sensor Chip NA:

Stock solution (2 M NaCl, 100 mM NaOH, 10 mL)

	Final concentration	Add:
NaCl	2 M	1.17 g
NaOH (1 M)	100 mM	1 mL
Ultrapure water		to 10 mL

Conditioning solution 1 (1 M NaCl, 10 mM HCl, 10 mL)

	Final concentration	Add:
NaCl	1 M	0.58 g
HCI (1 M)	10 mM	100 µL
Ultrapure water		to 10 mL

Conditioning solution 2 (1 M NaCl, 50 mM NaOH)

Mix the stock solution with ultrapure water 1:1 before use.

Wash solution (50% isopropanol, 1 M NaCl, 50 mM NaOH)

Mix the stock solution with isopropanol 1:1 before use. Use within a week.

Cleaning the flow system

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

Note: Perform any cleaning steps before docking Series S Sensor Chip NA.

Step	Action
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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.	

Preparations for use

Step	Action
1	Allow the sealed sensor chip pouch to equilibrate at room temperature for 15 to 30 minutes to prevent condensation on the chip surface.
2	Prepare the Biacore instrument with running buffer.
3	Open the sensor chip pouch. Make sure that the sensor chip support remains fully inserted into the sheath at all times.
4	Dock the sensor chip in the instrument as described in the <i>Operating Instruc-</i> <i>tions</i> .
	Note:
	Store undocked sensor chips in closed containers.

Immobilizing the ligand

Other Biacore systems

For Biacore systems other than Biacore 8K, and Biacore 4000, follow these steps to create an immobilization procedure on Series S Sensor Chip NA.

Step	Action
1	Dock Series S Sensor Chip NA as a custom chip.
2	In the immobilization setup, select Chip type →Custom and Custom Method , and then select New .
3	Construct the sequence of injections for the immobilization method including recommended conditioning and washing steps outlined in this instruction.

Biacore 8K

For Biacore 8K, follow these steps to create an immobilization procedure on Series S Sensor Chip NA.

Step	Action
1	Dock Series S Sensor Chip NA as a custom chip.
2	On the immobilization setup, select Chip type \rightarrow Custom \rightarrow Enter Custom Method.
3	Change the name of the assay step into NA biotin.
4	Remove all commands except <i>Ligand</i> .
5	Click Add command to construct the sequence of injections for the immobi- lization method, including recommended conditioning and washing steps outlined below.
6	Select FC2 , activate/deactivate in 1 to make sure that conditioning solution passes over both flow cells 1 and 2.

Biacore 4000

For Biacore 4000, follow the recommended steps to create an immobilization procedure on Series S Sensor Chip NA.

Step	Action	
1	Dock Series S Sensor Chip NA as a Series S Sensor Chip SA.	
2	Run a hydrodynamic addressing procedure as described in <i>Hydrodynamic addressing in Biacore 4000 when using pre-immobilized sensor chips (29214397)</i> available from cytiva.com/biacore.	
3	On Method Builder , set up a run including the conditioning procedure outlined in section <i>Conditioning, on page 6</i> . Let the injections pass over all spots (whole flow cell).	
4	Perform ligand immobilization according to the immobilization method for Series S Sensor Chip SA, but fill the wells intended for conditioning (marked as 1M NaCl 0.05M NaOH) with running buffer.	
Note:	For Biacore 4000 the maximum possible contact time is 360 s when Series S Sensor Chip NA has been docked as Series S Sensor Chip SA.	

Conditioning

Condition the sensor surface before ligand immobilization in order to reduce baseline drift and increase assay performance. The conditioning procedure should follow the sequence of injections reported in the following table. Make sure that conditioning is performed on both the active and the reference surface.

Injection	Solution	Contact time	Flow rate
1	Conditioning solution 1	60 s	10 µL/min
2	Conditioning solution 1	60 s	10 µL/min
3	Conditioning solution 2	60 s	10 µL/min

Immobilization

Typical injection parameters for the immobilization of a biotinylated ligand are:

- contact time = 600 s
- flow rate = 5 µL/min

Adjust the contact time depending on the desired coupling level. The coupling level depends on degree of biotinylation and concentration of the ligand. The contact time has to be determined for every ligand separately during assay development. As a guideline, use a contact time from 120 to 1200 s.

Use a low flow rate to reduce consumption of ligand.

For oligonucleotide ligands, immobilization solution should contain NaCl at a concentration of 0.5 M or higher.

Extra wash

After each ligand injection, run a wash using the wash solution and selecting **Wash** or **Extra wash** command according to the Biacore system used. The wash solution does not pass over the sensor surface.

Interaction analysis

Interaction analysis is performed by injection of samples over the sensor chip surface. Analyte molecules in the injected sample bind directly to the captured ligand.

In order to minimize drift, run at least 5 start-up cycles before the interaction analysis. For kinetic analysis, run at least 10 start-up cycles. Start-up cycles are run with sample replaced with running buffer. For details on experimental protocols and methodology, refer to Biacore handbooks and *cytiva.com/biacore*.

Run conditions

Running buffer

Series S Sensor Chip NA is compatible with all Cytiva running buffers used with Biacore systems

Analysis temperature

Series S Sensor Chip NA is designed for use at 25°C.

Start-up cycles

For best assay performance, run 1 to 3 start-up cycles using sample or buffer as analyte and identical settings as for the analysis cycles.

Regeneration

Regenerate the surface by removing the analyte from the captured ligand. Conditions should be chosen to achieve complete dissociation of the analyte without affecting the binding characteristics of the ligand. The surface of Series S Sensor Chip NA is resistant to some agents for this purpose (see following section for more information). The choice of regeneration procedure may be limited by the stability of the ligand.

Series S Sensor Chip NA is sensitive to acidic conditions and regeneration involving acidic pH should be avoided.

For more detailed information on regeneration strategies, refer to *Biacore Sensor Surface Handbook (BR100571)*.

Chemical resistance

The surface of Series S Sensor Chip NA has been tested with one minute pulses of some commonly used agents.

Agent	Concentration	Resistance
MgCl ₂	3 M	R
NaCl	5 M	R
NaOH/NaCl	50 mM/1 M	R
SDS	0.05%	R
Surfactant P20	0.5%	R
Acetonitrile (in 100 mM NaOH)	30%	NR
Glycine-HCl pH 1.5	10 mM	NR

Legend: R = Resistant; NR = Not Resistant.

Note: Other agents and concentrations have not been tested.

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