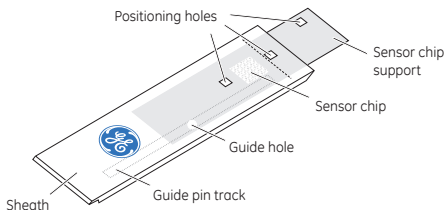


Sensor Chip L1

Product description

Order code:	BR-1005-43 (package of three sensor chips) BR-1005-58 (package of one sensor chip)
Storage:	The use-before date applies to chips stored at +2 to 8°C in unopened pouches.
Limitations of use:	Use of Sensor Chip L1 as recommended in Biacore 3000 and Biacore 2000 requires that Method Definition Language (MDL) is used. The recommended extra wash is not possible using the software wizards.



Note: *For in vitro use only.*



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

Application areas

Sensor Chip L1 is designed for interaction analysis in Biacore systems. The surface consists of a carboxymethylated dextran matrix preimmobilized with lipophilic groups for rapid and reproducible capture of lipid membrane vesicles with and without membrane proteins.

The binding process involves diffusion of the vesicles to the surface and incorporation of the lipophilic structures on Sensor Chip L1 into the lipid membrane, non-covalently anchoring the vesicle.

These instructions describe recommended procedures for lipophilic capture of membrane vesicles. The surface chemistry also allows covalent coupling to be used, based on carboxymethyl chemistry¹. For advice on covalent coupling procedures, refer to *Biacore Sensor Surface Handbook*.

Refer to www.gelifesciences.com/biacore for up-dates on applications and scientific publications.

Required solutions

See table below for solutions required for use with Sensor Chip L1.

Solution	Description
Running buffer	HBS-N (available from GE Healthcare) or other detergent-free buffer
Regeneration solution, for stripping vesicles off the chip	A detergent (e.g., 20 mM CHAPS ² or 40 mM OG ³) or a mixture of 2 parts isopropanol and 3 parts 50 mM NaOH.

¹ Karlsson, R. & Löfås, S., *Analyt. Biochem.* (2002) 300, 132-138

² 3-[[3-cholamidopropyl]dimethylammonio]-1-propane-sulfonate in water

³ n-octyl β -D-glucopyranoside in water

Preparations for use

- 1 If working in a humid environment, allow the sealed sensor chip pouch to equilibrate at room temperature for 15 to 30 minutes in order to prevent condensation on the chip surface.
- 2 Prepare the Biacore instrument with detergent-free running buffer. The buffer should be filtered (0.22 μm), and degassed for systems that do not have an integrated buffer degasser.
- 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 instrument handbook. Sensor chips that are not docked in the instrument should be stored in closed containers.

Capture of lipid vesicles

- 1 Prepare vesicles in running buffer. A concentration of 0.5 mM with respect to phospholipid is usually sufficient.
- 2 Condition the surface with two 30-second injections of detergent (e.g., 20 mM CHAPS or 40 mM OG) or a mixture of 2 parts isopropanol and 3 parts 50 mM NaOH before using the surface.
- 3 Inject the vesicle sample using a low flow rate (recommended 2 to 10 $\mu\text{l}/\text{min}$). Vesicle binding typically takes about 3 to 15 min.

Note: *The time required may vary according to vesicle composition, temperature and buffer conditions.*

- 4 Examine the sensorgram to monitor the adsorption of vesicles to the surface. The process is complete when the sensorgram flattens out at a constant response.
- 5 If compatible with the vesicle composition, one short pulse of 10 to 100 mM NaOH and/or regeneration solution may be injected after vesicle binding to stabilize the baseline before interaction analysis.

Refer to Biacore Sensor Surface Handbook for more detailed information on immobilization strategies and procedures.

Interaction analysis

Interaction analysis is performed as sample is injected over the sensor chip surface. Should non-specific binding occur, this may in some cases be successfully counteracted by coating the surface with an injection of serum albumin or other protein that does not interact with the analyte (recommended 0.2 mg/ml in running buffer) prior to analysis.

Refer to Biacore handbooks and www.gelifesciences.com/biacore for details on experimental protocols and methodology.

Regeneration

Removal of vesicles

The vesicles may be stripped from Sensor Chip L1 using a 30-second injection of detergent (e.g., 20 mM CHAPS or 40 mM OG) or isopropanol: 50 mM NaOH at a ratio 2:3. With this approach, fresh vesicles are captured for each cycle after the detergent is washed out of the system. To prevent carry-over of vesicles an extra wash (see *Appendix: Sample cycles with extra wash, on page 6*) using regeneration solution after the vesicle injection may be included in the cycle. This wash does not pass over the surface.

Removal of analyte

Regeneration of the vesicles on the surface may be performed by selective dissociation of the bound analyte. Conditions should be chosen to achieve complete dissociation of the analyte without affecting the binding characteristics of the vesicle. Agents that will not destabilize captured POPC⁴ vesicles are listed in the following table (see *Chemical resistance, on page 5*). Vesicles with other compositions may require different regeneration procedures.

Detergents and organic solvents may alter or destabilize vesicle binding or even cause it to disintegrate. These type of agents may be used to strip the vesicles from the sensor chip.

Refer to Biacore Sensor Surface Handbook for more detailed information on regeneration strategies.

⁴ 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine

Chemical resistance

The surface of Sensor Chip L1 is resistant to 1-minute pulses of many commonly used agents. See table below for information of common agents compatible with Sensor Chip L1 and POPC vesicles. Agents for which no concentration is listed have not been tested.

Agent	Compatible with the sensor surface	Compatible with POPC vesicles
Acetonitrile	30%	-
DMSO	10%	10%
DTE	0.1 M	-
EDTA	0.35 M	-
Ethanol	70%	10%
Ethanolamine	1 M	-
Ethylene glycol	100%	-
Formamide	40%	-
Formic acid	20%	-
Glycine pH 1.5 to 3.0	100 mM	-
HCl	100 mM	100 mM
Imidazole	300 mM	-
MgCl ₂	4 M	-
NaOH	100 mM	100 mM
NaCl	5 M	-
SDS	0.5%	-
Surfactant P20	5%	-
Urea	8 M	-

Appendix: Sample cycles with extra wash

Follow the instructions below to add and run extra washes when capturing and removing vesicles in each sample cycle.

In Biacore X100:

Create a method that includes the following steps:

Step	Description
Surface conditioning	Condition the surface with two 30-second injections of regeneration solution before using the surface. Flow rate 10 $\mu\text{l}/\text{min}$ is recommended. This step is used only in the first cycle of each run.
Extra wash with buffer	Use the Extra wash command with running buffer.
Capture of lipid vesicles	Inject the vesicle sample using a low flow rate (recommended 2 to 10 $\mu\text{l}/\text{min}$). Vesicle binding typically takes 3 to 15 min.
Additional wash using regeneration solution	Use the Extra wash command with regeneration solution after vesicle injection. This solution does not pass over the surface.
Injection of sample	Sample injection, injection times and flow rates depend on the application.
Regeneration	Vesicles may be stripped from Sensor Chip L1 using a 30-second injection of regeneration solution. Low flow rate (5 to 10 $\mu\text{l}/\text{min}$) can be used.
Extra wash with buffer	Use the Extra wash command with running buffer.

In Biacore 3000 and Biacore 2000:

- From www.biacore.com/applicationsupporttools - Methods, download the appropriate Method Definition Language (MDL) method
or
- Create an MDL method that includes the following steps:

Command	Procedure
Surface conditioning	Condition the surface with two 30-second injections of regeneration solution before using the surface. Flow rate 10 $\mu\text{l}/\text{min}$ is recommended. This step is used only in the first cycle of each run.
Extra wash with buffer	Use the Extraclean command.
Capture of lipid vesicles	Inject the vesicle sample using a low flow rate (recommended 2 to 10 $\mu\text{l}/\text{min}$). Vesicle binding typically takes 3 to 15 min.
Additional wash with regeneration solution	Wash with regeneration solution after vesicle injection. This solution does not pass over the surface. Enter the wash commands in the following order: <ol style="list-style-type: none">1 WASHPOS n r1a1 (needle will be washed with wash solution from a defined position).2 WASHPOS s r1a1 (sample loop will be washed with wash solution from a defined position).3 WASH n (needle will be washed with running buffer).4 WASH s (sample loop will be washed with running buffer).
Injection of sample	Sample injection, injection times and flow rate depend on the application.
Regeneration	Vesicles may be stripped from Sensor Chip L1 using a 30-second injection of regeneration solution. Low flow rate (5 to 10 $\mu\text{l}/\text{min}$) can be used.
Extra wash with buffer	Use the Extraclean command.

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