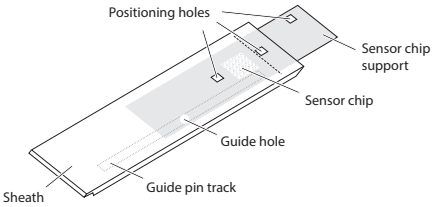
Sensor Chip HPA

Instructions for Use

Product description

Product code:	BR100030 (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 support sheath. Each cassette, consisting of a sensor chip and sheath assembly, is individually packed under a nitrogen atmosphere in a sealed pouch.

Note: *For in vitro use only.*

Application areas

Sensor Chip HPA is designed for interaction analysis in Biacore™ systems. The flat hydrophobic surface consists of long-chain alkane thiol molecules attached directly to a gold film. User-prepared liposomes adsorb spontaneously to the surface to form a supported lipid monolayer, with polar head groups directed out towards the solution. Molecules associated with membrane surfaces can be incorporated into the monolayer and used as ligands.

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

Preparation for use

Biacore instrument

Make sure that the Biacore instrument is clean prior to docking Sensor Chip HPA. In addition to regular maintenance, it is important to remove all detergent from the flow system. Trace amounts of detergents might otherwise bind to the chip and interfere with the formation of lipid structures on the surface.

Follow the washing schedule below before docking the chip.

Step	Action
1	Run ***Desorb*** followed by ***Sanitize***. Use Sensor Chip Maintenance for this step.
2	Switch from running buffer to water using the procedure for buffer change provided in the instrument software or described in the *Instrument Handbook*.
3	Run the instrument at a low flow rate (5 to 10 µl/min) overnight.
4	Switch to detergent-free running buffer for the experiments, again using the regular procedure for buffer change. The buffer should be filtered (0.22 µm), and degassed for systems that do not have an integrated buffer degasser.

Sensor Chip HPA

Step	Action
1	If you are 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	Open the sensor chip pouch. To protect the chip from dust particles, make sure that the sensor chip support remains fully inserted into the sheath at all times.
3	Dock Sensor Chip HPA in the instrument as described in the *Instrument Handbook*. ***Note:*** *Sensor chips that are not docked in the instrument should be stored in closed containers.*

Adsorption of lipid monolayers

Step	Action
1	Prepare liposomes in running buffer. A concentration of 0.5 mM with respect to phospholipid is commonly sufficient. Smaller liposomes will adsorb faster to the surface and give better surface coverage.
2	Wash the surface with a 5-minute injection of 40 mM octyl glucoside (n-octyl β-D-glucopyranoside) in water.
3	Inject the liposome sample directly after this wash using a low flow rate (2 to 10 µl/min). The process of coating Sensor Chip HPA with a lipid monolayer typically takes 0.5 to 3 hours depending on temperature, size and composition of liposomes, and running buffer.
4	Examine the sensorgram to monitor the adsorption of liposomes to the surface. The process is complete when the sensorgram flattens out at a constant response. It is important that the sensor surface is completely coated with lipid monolayer, since the exposed surface on Sensor Chip HPA has a strong tendency to bind proteins non-specifically. Complete coverage is represented by a response of 2000 to 2500 to RU above baseline. If the response is lower than this, try injecting liposomes or protein that do not interact with the analyte to cover the remaining surface.
5	At this stage the surface might carry multiple lipid layers and partially fused liposomes. If compatible with the lipid preparation, wash the surface with one short (0.5 to 1 min) injection of 10 to 100 mM NaOH. Alternatively, the flow rate can be increased briefly (e.g., 30 to 50 µl/min for 2 to 3 min) to wash away loosely attached lipid structures. The response level should be stable after washing.

Interaction analysis

The lipid monolayer covering the surface participates directly in the interaction under study. Interaction analysis is performed as analytes in solution are injected in sequence over the surface of Sensor Chip HPA. Use detergent-free buffers that have been thoroughly degassed. Microscopic air bubbles in the buffer have a strong tendency to stick to the hydrophobic surface of the sensor chip. Running experiments at 25°C or below can also help to reduce bubble formation.

Refer to Biacore handbooks and *cytiva.com/biacore* for details on experimental protocols and methodology.

Regeneration

Regeneration involves removing analyte from the surface, leaving the lipid monolayer intact for the next analysis cycle. Lipid monolayers composed of e.g., DMPC or POPC are quite robust in this respect and can withstand exposure to 100 mM NaOH and 100 mM HCl. However, the sensitivity of the user-defined binding sites on the ligand will ultimately determine the regeneration agents that can be used.

Do not use detergents or organic solvents for regeneration unless you want to remove the lipid monolayer.

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

Chemical resistance

The chemical resistance of Sensor Chip HPA in practical applications is determined by the properties of the lipids covering the surface.

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