Step 3: Inject analytes

Analytes are typically injected in the horizontal direction perpendicular to the ligand (lipid assemblies). For kinetic analysis, analyte injections are usually performed at a high flow rate (100 µl/min) to avoid mass transport limitation, but lower flow rates may be used to reduce sample consumption. The injection conditions — including association and dissociation time, flow rate, and analyte concentrations — should be optimized in order to obtain high-quality interaction analysis.

Step 4: Regenerate the LCP sensor chip surface

The regeneration is accomplished by DNA dehybridization using the following options (injection details are listed in the tables below). Injection 2 is optional in both cases because it is used to remove the remaining lipid assemblies if the regeneration is incomplete with injection 1.

- Inject an 8 M urea solution freshly prepared in deionized water

Injection	Reagent	Orientation	Volume	Flow Rate
1	8 M urea	Vertical	150 µl	30 µl/min
2	20 mM CHAPS	Vertical	150 µl	30 µl/min

Note: Injection 2 is optional.

- In case urea is not available, inject deionized water

Injection	Reagent	Orientation	Volume	Flow Rate
1	Deionized water	Vertical	150 µl	30 µl/min
2	20 mM CHAPS	Vertical	150 µl	30 µl/min

Note: Injection 2 is optional.

Ordering Information

Catalog #	Description
176-5041	**ProteOn LCP Sensor Chip**, for capturing lipid assemblies such as liposomes; for use with the ProteOn LCP capturing reagent kit
176-2310	**ProteOn LCP Capturing Reagent Kit**, for capturing lipid assemblies such as liposomes; for use with the ProteOn LCP sensor chip
176-2361	**ProteOn Lipid Modification Conditioning Solution**, 45 ml, for cleaning and stabilizing the chip surface before capturing lipid assemblies such as liposomes
176-2300	**ProteOn Liposome Capturing Kit**, includes 1 ProteOn LCP sensor chip, 1 ProteOn LCP capturing reagent kit, and ProteOn lipid modification conditioning solution
176-4117	**ProteOn Postexperiment Cleaning Kit**, contains 50 ml 20 mM HCl and 50 ml 2% Contrad 70; for ProteOn system postexperiment cleaning
176-2520	**ProteOn Maintenance and Postexperiment Cleaning Kit**, contains 1 maintenance and 2 cleaning chips, 2 L 2% Contrad 70, 2 L 70% isopropyl alcohol, 50 ml 20 mM HCl, and 50 ml 2% Contrad 70; for ProteOn system maintenance and postexperiment cleaning

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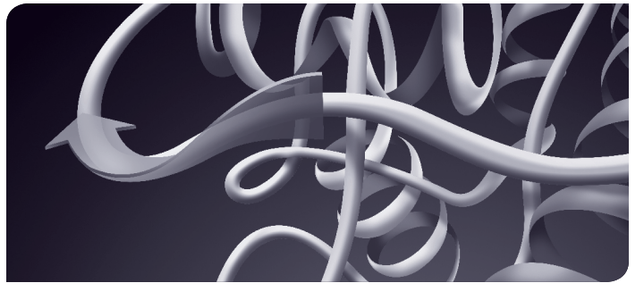

ProteOn™ Liposome Capturing Kit

BIO-RAD

ProteOn Liposome Capturing Kit

The ProteOn liposome capturing kit is designed for capturing lipid assemblies such as liposomes and lipoparticles. It is a tool for the interaction analysis of membrane proteins embedded in the lipid bilayer of these assemblies.

The kit is composed of a ProteOn LCP sensor chip, an LCP capturing reagent kit, and lipid modification conditioning solution. The LCP sensor chip surface is functionalized with NeutrAvidin in a planar configuration that is formed on a self-assembled monolayer. This surface can be activated by a biotinylated DNA tag from the LCP capturing reagent kit to capture DNA-labeled liposomes. The LCP capturing reagent kit is able to attach DNA tags to the liposomes and anchor them to the chip surface through DNA hybridization. Furthermore, the LCP capturing reagent kit enables the binding of two or more layers of lipid assemblies for additional sensitivity.

The lipid modification conditioning solution (20 mM CHAPS) is used to clean and stabilize the chip surface prior to capturing liposomes.

Surface regeneration can be accomplished by DNA dehybridization using either 8 M urea solution (preferred to achieve complete dehybridization) or deionized water. The urea solution is not provided in the kit because it should be prepared fresh before use for best results. In case of incomplete regeneration, it is recommended to remove the remaining lipid assemblies using the lipid modification conditioning solution. A recommended protocol for using the kit is described below.

Important notice: (1) PBS is a typical running buffer for the LCP sensor chip. PBST or other detergent-containing buffers should be avoided because the presence of detergent will affect liposome stability. (2) The ProteOn instrument should be maintained properly according to the user manual. For these applications, it is especially important to perform a post-experiment cleaning protocol after the use of each chip. (3) Following the instructions will provide at least 13 injections of biotin-ssDNA for surface activation, 26 injections for capturing of cholesterol-modified-dsDNA 1 (chol-dsDNA 1) labeled liposomes, and 21 injections of chol-dsDNA 2 for forming more than one liposome layer.

Step 1: Prepare the LCP capturing reagents

Stock solutions in the LCP capturing reagent kit

Add the following volumes of PBS to the vials to dissolve the oligonucleotides. It is recommended to spin down the solid material before opening the vials (for example, by centrifugation between 1,000 and 6,000 rpm for 1 min). Make sure that all the material is dissolved by applying PBS to the entire inner vial surface and vortexing thoroughly.

1. Biotin-ssDNA: add 650 µl of PBS to prepare a 4 µM solution.
2. Chol-ssDNA 1S: add 325 µl of PBS to prepare a 4 µM solution.
3. Chol-ssDNA 1L: add 325 µl of PBS to prepare a 4 µM solution.
4. Chol-ssDNA 2S: add 325 µl of PBS to prepare a 4 µM solution.
5. Chol-ssDNA 2L: add 325 µl of PBS to prepare a 4 µM solution.

Store these stock solutions at –20°C or use them immediately for hybridization. It is recommended to use molecular biology grade reagents for the reconstitution.

Hybridization

1. Mix the entire volume of chol-ssDNA 1S stock solution (325 µl) with the entire volume of chol-ssDNA 1L stock solution (325 µl). Incubate for 30 min at room temperature. Store the 2 µM chol-dsDNA 1 stock solution at –20°C.
2. Similarly, mix the entire volume of chol-ssDNA 2S stock solution (325 µl) with the entire volume of chol-ssDNA 2L stock solution (325 µl). Incubate for 30 min at room temperature. Store the 2 µM chol-dsDNA 2 stock solution at –20°C.

Before long-term storage, it is recommended to aliquot the chol-dsDNA solutions into at least two equal volumes to avoid multiple freeze-thaw cycles. The chol-dsDNA solutions can be kept at room temperature for experiments planned for the same day only, but they should be stored at –20°C for long-term use.

Step 2: Capture lipid assemblies (liposomes)

Preparation of reagents

- Thaw the required stock solutions. Take the required volume for use, and store the rest back at –20°C. The required volumes can be calculated using the instructions below

Important notice: Add 100 µl of void volume per injection.

- Dilute the chol-dsDNA 1 stock solution fivefold with PBS to a final concentration of 0.4 µM
- If chol-dsDNA 2 is required for the experiment, dilute the stock solution fivefold with PBS to a final concentration of 0.4 µM

Labeling of liposomes

- Mix equal volumes of 0.4 µM chol-dsDNA 1 solution and 2 mg/ml liposome solution, making the final concentrations 0.2 µM and 1 mg/ml, respectively. Incubate the mixture for 30 min at room temperature to allow the cholesterol moieties of the DNA tags to incorporate into the lipid bilayer of the liposomes

Important notice: Long-term storage of the labeled liposomes is not recommended. Prepare a volume sufficient only for experiments planned for the same day.

- Dilute the biotin-ssDNA stock solution threefold with PBS to a final concentration of 1.3 µM to a volume that will be sufficient for the planned experiments

Capturing of liposomes

(injection details are listed in the table below)

- Inject the biotin-ssDNA solution for surface activation
- Precondition the chip surface with the lipid modification conditioning solution (20 mM CHAPS) before liposome capturing
- Inject the chol-dsDNA 1 tagged liposome solution
- If an additional liposome layer is to be produced, inject the chol-dsDNA 2 solution (0.4 µM). Allow the signal to stabilize for 5 min. Then inject the chol-dsDNA 1 tagged liposome solution. This step may be repeated to form multiple liposome layers

Injection	Reagent	Orientation	Volume	Flow Rate
1	1.3 µM biotin-ssDNA	Vertical	50 µl	30 µl/min
2	20 mM CHAPS	Vertical	150 µl	30 µl/min
3	1 mg/ml chol-dsDNA 1 tagged liposomes	Vertical	150 µl	30 µl/min
4	0.4 µM chol-dsDNA 2	Vertical	50 µl	30 µl/min
5	1 mg/ml chol-dsDNA 1 tagged liposomes	Vertical	150 µl	30 µl/min

Note: Injections 4 and 5 are optional.