Step 3: Inject analytes

Analysys 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 table 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
- In case urea is not available, inject deionized water

Note: 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.

Ordering Information

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>176-2300</td>
<td>ProteOn Liposome Capturing Kit, includes 1 ProteOn LCP sensor chip, 1 ProteOn LCP capturing reagent kit, and ProteOn lipid modification conditioning solution</td>
</tr>
<tr>
<td>176-4117</td>
<td>ProteOn Postexperiment Cleaning Kit, contains 50 mL 20 mM HCl and 50 mL 2% Contrad 70</td>
</tr>
<tr>
<td>176-2520</td>
<td>ProteOn Maintenance and Postexperiment Cleaning Kit, contains 1 maintenance and 2 cleaning chips, 2 L 2% Contrad 70 and 2 L 70% isopropyl alcohol, 50 mL 20 mM HCl, and 50 mL 2% Contrad 70; for ProteOn system maintenance and postexperiment cleaning</td>
</tr>
</tbody>
</table>

NeutrAvidin is a trademark of Thermo Fisher Scientific Inc.
The 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 recognizes biotinylated DNA tag from the LCP capturing reagent kit. This surface can be activated by 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 liposomes and anchor them to the chip surface through DNA hybridization.

Furthermore, the LCP capturing reagent kit enables the formation of two or more layers of lipid assemblies for additional sensitivity.

**The lipid modification conditioning solution**

The lipid modification conditioning solution (0.2 mM CHAPS) is used to clean and stabilize the chip surface prior to capturing liposomes. Surface regeneration can be accomplished by DNA desorption after use or by dilution with water. The chip surface is reactivated by applying the DNA to the inner surface of the chip in the LCP sensor chip kit. 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 liposomes and anchor them to the chip surface through DNA hybridization.

Furthermore, the LCP capturing reagent kit enables the formation of two or more layers of lipid assemblies for additional sensitivity.

1. Biofilm: add 650 µl of PBS to prepare a 4 µM solution.
2. Chol-ssDNA 1L stock solution (325 µl) with the entire volume of chol-ssDNA 1 stock solution at –20°C.
3. Chol-ssDNA 1S stock solution (325 µl). Incubate for 30 min at room temperature.
4. Chol-ssDNA 2L stock solution (325 µl) with the entire volume of chol-ssDNA 2 stock solution at –20°C. Incubate for 30 min at room temperature.
5. Chol-ssDNA 2S stock solution (325 µl) with the entire volume of chol-ssDNA 2 stock solution at –20°C. Incubate for 30 min at room temperature.
6. Chol-ssDNA 2L stock solution (325 µl) with the entire volume of chol-ssDNA 2 stock solution at –20°C. Incubate for 30 min at room temperature.
7. Chol-ssDNA 2S stock solution (325 µl) with the entire volume of chol-ssDNA 2 stock solution at –20°C. Incubate for 30 min at room temperature.
8. Chol-ssDNA 1L stock solution (325 µl). Incubate for 30 min at room temperature.
9. Chol-ssDNA 1S stock solution (325 µl). Incubate for 30 min at room temperature.
10. Chol-ssDNA 1L stock solution (325 µl). Incubate for 30 min at room temperature.
11. Chol-ssDNA 1S stock solution (325 µl). Incubate for 30 min at room temperature.
12. Chol-ssDNA 1L stock solution (325 µl). Incubate for 30 min at room temperature.
13. Chol-ssDNA 1S stock solution (325 µl). Incubate for 30 min at room temperature.
14. Chol-ssDNA 1L stock solution (325 µl). Incubate for 30 min at room temperature.
15. Chol-ssDNA 1S stock solution (325 µl). Incubate for 30 min at room temperature.
16. Chol-ssDNA 1L stock solution (325 µl). Incubate for 30 min at room temperature.
17. Chol-ssDNA 1S stock solution (325 µl). Incubate for 30 min at room temperature.
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19. Chol-ssDNA 1S stock solution (325 µl). Incubate for 30 min at room temperature.
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