

Application Note # 130

SPR Navi™ Immobilizer

SPR Navi™ Immobilizer is a small stand-alone device for functionalization of sensor slides used in SPR Navi™ Multi Parametric Surface Plasmon Resonance (MP-SPR) instruments.

Features

Compatibility. SPR Navi™ Immobilizer is compatible with the SPR Navi™ sensor slides and SPR Navi™ sensor slide holder. The immobilizer forms **two channels**, which copy the shape of the SPR Navi™ standard flow-cell (Fig. 1). Liquid reagents can be inserted in the chambers with a syringe or a pump via tubing connection.

Extended immobilization temperature. The SPR Navi™ Immobilizer is portable and thus, can be also placed on a hot plate or in an incubator, for instance. Immobilization on a hot plate enables reactions at higher or lower temperatures than those possible *in situ* using SPR Navi™ instruments.

Low reagent consumption. The immobilizer enables *ex situ* immobilization with minimized reagent consumption. After immobilization, only the channel area is covered with the reagents. While *ex situ* immobilization on a Petri-dish requires covering the whole sensor slide area (240 mm²), the *ex situ* immobilization with SPR Navi™ Immobilizer requires covering of channel area only (24 mm²). The total internal volume of the device is a few tens of microliters, allowing small volumes to be used.

Save instrument time. The *ex situ* sensor preparation with an external SPR Navi™ Immobilizer can save instrument time from immobilization to actual measurements. An especially useful property, when the SPR Navi™ instrument is in heavy use.

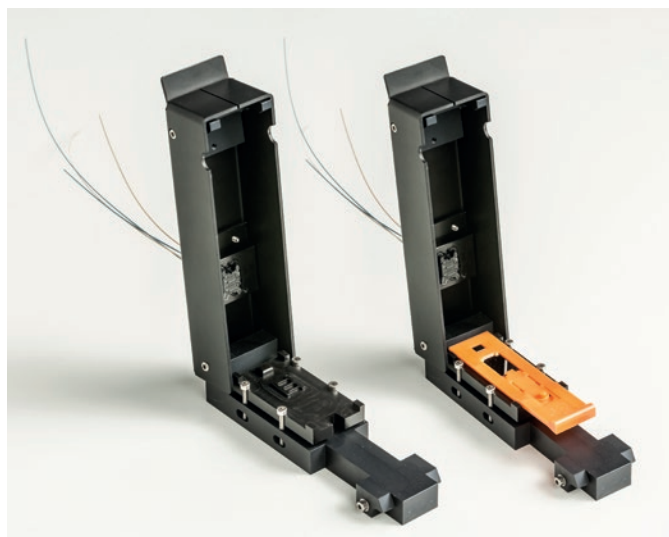


Figure 1. SPR Navi™ Immobilizer. Loading of sensor into the immobilizer.

Preparation	Immobilization strategy	Immobilization temperature	Surface immobilization	Reagent consumption
<i>In situ</i>	SPR Navi™ 200, 210A, 220A	25-45°C ^(a)	12 or 24 mm ² ^(b)	Medium
<i>Ex situ</i>	Petri dish or similar	Sample dependent	240 mm ²	High
<i>Ex situ</i>	SPR Navi™ Immobilizer	Sample dependent	12 or 24 mm ² ^(b)	Low

Note:

(a) Temperature range depends on the room temperature.

(b) For a single channel, the surface area is 12 mm². For both channels, the total is 24 mm².

Procedure

Place the SPR Navi™ sensor holder with the sensor slide into the immobilizer. Make sure that the sensor is facing with the metal side up and close the device (Fig. 1). Remember to use same cleaning protocols as in other reactions in beakers or *in situ* reactions to ensure best results. The immobilizer has two flow channels, and each of the channels can be handled separately or they can be connected in series. Liquid can be put through the channels with a syringe or using a pump (Fig. 2). Immobilization can include many steps and different liquids, if needed. Interaction time with sensor can be from seconds to hours depending on the protocol at hand. After the functionalization procedure, the sensor is easy to remove and is ready for a measurement. The immobilizer can be cleaned with same procedures as SPR Navi™ instruments.

For further self assembly instructions, please refer to your chemicals supplier or to the general literature. For further information about biosensor immobilization protocols, see for instance:

<http://sprpages.nl/experiments/immobilization.html>

Storage of immobilized sensors

It is possible to store the sensors for later use. The sensors that you removed from the SPR Navi™ can be used immediately or can be placed into the same plastic boxes, in which SPR Navi™ sensors arrive. Remember also to mark the lid of the box. The storage conditions depend on the sample functionalized on the sensor, but most commonly:

- Wet, in a solution compatible with the sensor chip and ligand
- Dry, desiccated in a nitrogen environment

The immobilized sensors can be also re-used (depending on application). For storage after use, clean the sensor well inside the SPR Navi™ instrument. Remove all bound sample analytes such as lipids and proteins. First, clean the sensor *in situ* with a running buffer and possibly some antibacterial agent.

Example

The immobilizer has been successfully used to prepare dNA-thiol self-assembled monolayer (SAM). A cleaned sensor slide (Au) was placed in the SPR Navi™ sensor holder, which was placed in the SPR Navi™ Immobilizer. dNA-thiol was dissolved in pure water (concentration 4 µg/ml), and an excess amount of 50 µl was used to treat one of the flow channels in order to make an active coating. The interaction time was 15 minutes, and the surface was washed with 1 ml of pure water after the dNA treatment. Next, both flow channels were treated with N,N-bis (2-hydroxyethyl)-α-lipoamide 1mM in methanol for 15 minutes in order to prevent non-specific interactions in later experiment. The whole procedure resulted with an active dNA sensor with one active and one reference channel.

The sensor was further used in measuring interactions of oligonucleotides. Oligonucleotides with concentrations ranging from 0,1-1000 nM were successfully detected and quantified with high sensitivity and precision using the in SPR Navi™ Immobilizer-functionalized sensor and SPR Navi 200 (Fig. 3).

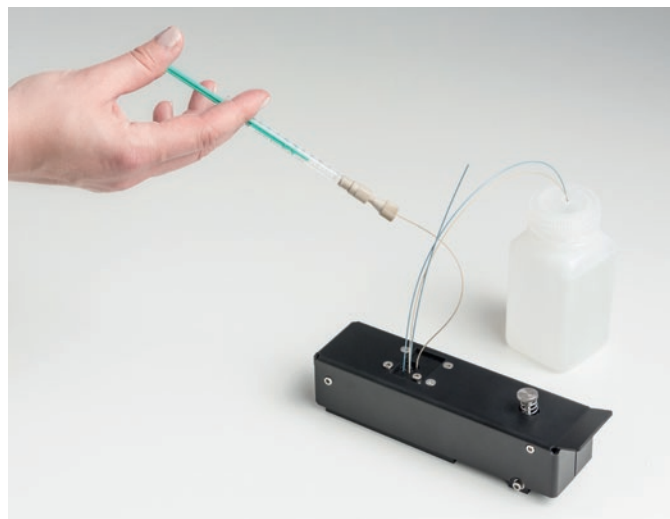


Figure 2. SPR Navi™ Immobilizer. Loading of one channel.

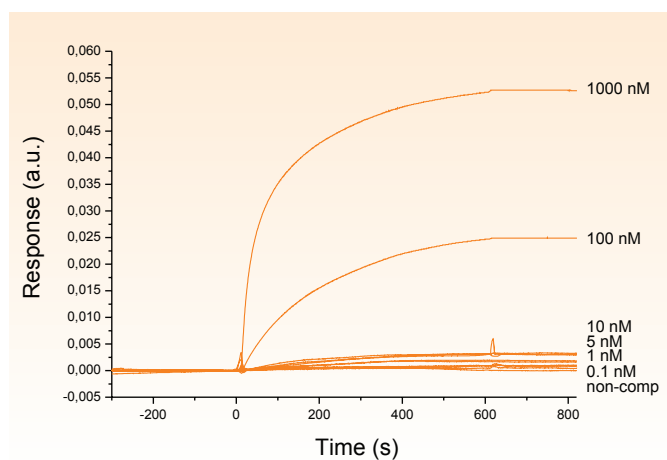


Figure 3. A compilation of the all the injections used in the experiment, concentrations ranging from 1000 nM to 0.1 nM.