TECH GUIDE
PROTEIN A SENSORS KIT

Immobilization method:	Capture
Ligand Requirements:	IgG derived antibody
Ligand orientation:	Oriented via Fc portion of IgG

Kit Shelf life	6 months

Overview

Protein A is used to capture the Fc region of IgG antibodies for directional antibody immobilization allowing the variable region to be oriented away from the sensor surface. The Protein A Sensor Kit contains all reagents necessary to create a Protein A functionalized surface over a Carboxyl sensor (Figure 1). This coupling method results in stable attachment of antibody ligands to the surface such that binding interactions can be easily measured in the OpenSPR instrument.

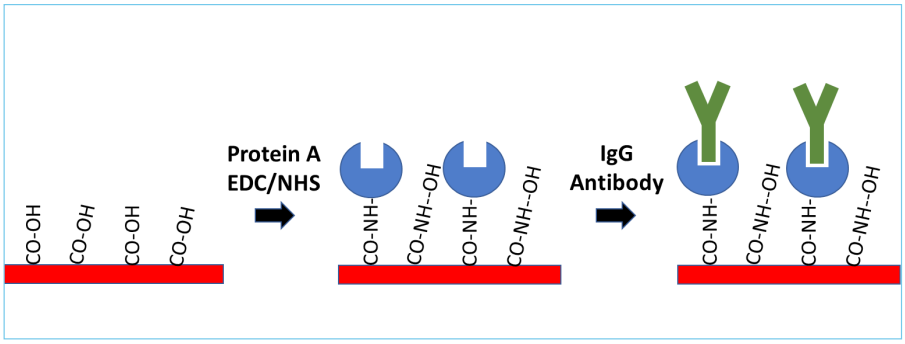

Figure 1. Covalent coupling of Protein A to Carboxyl Sensor chip (Step 1), followed by Antibody immobilization to Protein A (Step 2).

Kit Contents

	Storage Buffer	Recommended Storage
Carboxyl Sensors	PBS	4 °C
Protein A aliquots	HBS-P	-20 °C*
Protein A Coupling Kit	N/A	4 °C

* Avoid freeze-thaw cycles

Materials and Reagents Required for Coupling:

- Carboxyl Sensor
- Protein A Aliquot
- 10 mM HCl, pH 2-3
- Protein A Coupling Kit
  - EDC/NHS
  - Protein A immobilization buffer
  - Blocking solution

Injection Volumes

Minimum recommended injection volumes recommended for a 100 µL sample loop:

OpenSPR Rev 4	150 µL
OpenSPR-XT Rev 4	200 µL
OpenSPR Rev 3	200 µL
OpenSPR-XT Rev3	300 µL

Protein A Coupling Kit Preparation

Preparation of EDC aliquots

1. Dissolve provided EDC into 1 mL activation buffer.
2. Pipette 10 aliquots of 100 µL and store at –20°C.

Preparation of NHS aliquots

1. Dissolve provided NHS into 1 mL activation buffer.
2. Pipette 10 aliquots of 100 µL and store at –20°C.

Preparation of Protein A Solution

1. Immediately before use in the experiment, thaw one aliquot of Protein A and dilute it to 200 µL with the Protein A Immobilization Buffer. It is recommended to minimize the incubation time of the Protein A in the immobilization buffer.

Buffer Conditions

Conditions to avoid:

- Tris buffers
- Strong nucleophiles (eg. sodium azide)
- Protein additives (eg. BSA)

Running buffers with primary amine groups, strong nucleophiles or protein additives must be avoided for amine coupling as these will compete with the ligand immobilization. If buffer components containing primary amine groups are required for analyte analysis, it is recommended to switch the running buffer after the immobilization protocol is complete.

Ligand Removal

Removal of the protein A capturing molecule that has been covalently-coupled to the sensor carboxyl groups is not possible. Removal of a captured ligand (e.g. IgG) from protein A is possible. This can be done by using Glycine HCl at pH 1.5-3 as the regeneration buffer to remove existing IgG from the protein A surface for binding other antibody samples.

Referencing

For the 2-Channel OpenSPR, it is recommended to immobilize the ligand in channel 2 only and use channel 1 without any ligand (EDC/NHS activation, followed by Protein A immobilization step and blocking solution only) as the reference channel. As an alternative, a negative control antibody can be bound to the reference sensor surface in channel 1. For a non-specific binding experiment using the 1-Channel OpenSPR, it is recommended to prepare the Protein A functionalized sensor surface and omit the antibody ligand immobilization step. This will simulate the open sites on the ligand-bound Protein A sensor. Next, inject an analyte at the highest concentration to be used for the experiment. As an alternative, a negative control antibody can be bound to the sensor surface. Immobilization of the ligand could then be performed on this surface thereafter.

Additional Notes

If the analyte is also an antibody it will get captured by the Protein A in channel 1 as well as bind to the ligand in channel 2, thus it would be advisable to use a different coupling method for the ligand, such as Amine Coupling.

COUPLING PROCEDURE

1. Surface Conditioning

Perform an injection of 10 mM HCl (pH 2-3) to clean the sensor surface.

CH	Flow Rate
1+2	150 µL/min

2. Surface Activation

Thaw and immediately mix 1 aliquot of EDC and NHS together. *The EDC/NHS ester has a short half-life so it must be injected immediately after it is mixed.* Inject this mixture to activate the sensor (5-minute interaction time).

CH	Flow Rate
1+2	20 µL/min

3. Surface Preparation

Dilute the Protein A into 200 µL of the protein A immobilization buffer to create a concentration of 20 µg/mL.

Inject the Protein A Solution into the OpenSPR closely following the EDC/ NHS activation period (5-minutes of interaction time). You should expect at least 300 pm of Protein A immobilization.

CH	Flow Rate
1+2	20 µL/min

4. Blocking

Inject the Blocking solution to deactivate the remaining active carboxyl groups on the sensor (5 minute interaction time).

CH	Flow Rate
1+2	20 µL/min

5. Ligand Immobilization

Dilute the ligand (IgG antibody) to be immobilized in the running buffer to a concentration of 10-50 µg/mL. Inject the ligand into the instrument immediately following dilution (5-minute interaction time).

CH	Flow Rate
2	20 µL/min

Evaluation

The amount of ligand binding is calculated by comparing the signal after the protein A immobilization step to the signal after the ligand immobilization step. In the example shown in Figure 2, it is approximately 800 RU. Ensure this meets your minimum ligand immobilization target.

If your immobilization target is not reached, repeat another ligand immobilization injection, or consider optimization of this step.

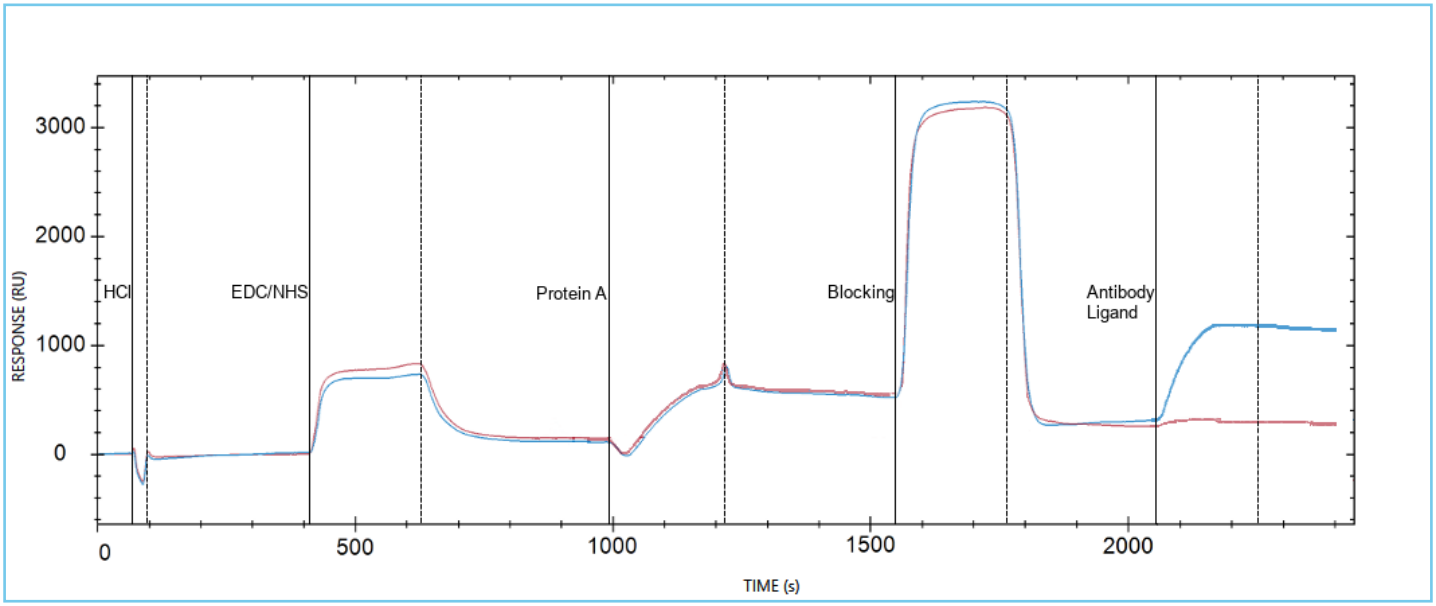

Figure 2. Example response graph covalently coupling Protein A to a Carboxyl sensor, followed by IgG ligand immobilization on the OpenSPR 2-channel system (red: Channel 1, blue: Channel 2).