Carboxyl Sensors

Tech Guide

Immobilization method:	Covalent
Ligand requirements:	Free -NH2 groups (eg. Lysine residues)
Ligand orientation :	Random
Recommended coupling kit:	Amine Coupling Kit - Proteins or Amine Coupling Kit - Small Molecules

Overview

The Carboxyl Sensors have a uniform layer of carboxyl (-COOH) groups on their surface that provide a foundation for covalent immobilization of protein ligands. These surface carboxyl groups are activated with 1-ethyl-3-(3-dimethylaminopropyl)- carbodiimide (EDC) and N-hydroxysuccinimide (NHS) to chemically couple the ligand via its primary amine groups (Figure 1). The remaining active carboxyl groups are then deactivated using a blocking solution to prevent further coupling and reduce non-specific binding.

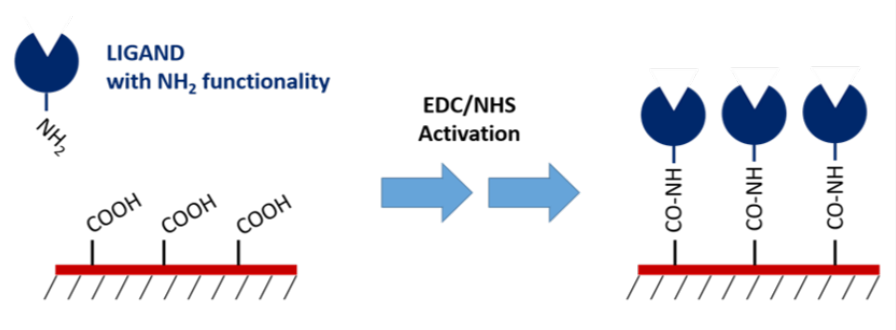

Figure 1. Covalent coupling of ligand to Carboxyl Sensor via EDC/NHS amine coupling.

Sensor storage buffer	PBS
Recommended storage	4°C
Shelf life	6 months

Materials and Reagents Required for Coupling:

- **Carboxyl Sensor**
- **10 mM HCl, pH 2-3**
- **Amine Coupling Kit**
  - EDC
  - NHS
  - Activation buffer
  - Immobilization buffer (for small molecules only)
  - 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

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.

Pre-Concentration of the Ligand

Large ligands such as proteins are usually immobilized to Carboxyl Sensors using a method known as pre-concentration. Pre-concentration uses electrostatic forces to increase the local concentration of ligand at the surface of the sensor. This is achieved by diluting the ligand into a buffer typically at least 0.5 pH units below the isoelectric point (pI) of the protein ligand, so that the protein carries a net positive charge. Nicoya provides an optimized Activation Buffer with the Amine Coupling Kit (Proteins) for pre-concentration for most proteins. If sufficient immobilization levels cannot be reached with the Activation Buffer, the ligand concentration can be increased, or further pre-concentration optimization can be performed using Nicoya's Immobilization Optimization Kit.

Ligands which typically cannot use the pre-concentration technique include:

- Small molecules
- Peptides, Nucleic Acids, DNA
- Carbohydrates
- Proteins with a pI below 3.5

For small, low charged species it is recommended to use Nicoya's Amine Coupling Kit for Small Molecules. Larger ligands that cannot be used for pre-concentration (eg. highly acidic proteins) should be immobilized through other methods such as capture coupling (Streptavidin-Biotin or 6x Histidine tag-NTA).

Ligand Removal

Removal of a ligand covalently coupled to a Carboxyl Sensor surface is not possible.

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 blocking solution only) as the reference channel. As an alternative, a negative control protein can be bound to the reference sensor surface in channel 1 after the EDC/NHS activation. For a non-specific binding experiment using the 1-Channel OpenSPR, it is recommended to prepare a deactivated sensor surface without any ligand (EDC/NHS activation followed by blocking solution only). This will deactivate and block the active carboxyl groups, simulating the open sites on the ligand-bound sensor. Next, inject an analyte at the highest concentration to be used for the experiment. Immobilization of the ligand cannot be performed on this surface thereafter. As an alternative, a negative control protein can be bound to the sensor surface after the EDC/NHS activation.

Additional Notes

Most proteins contain several amine groups so that efficient attachment can be achieved without seriously affecting the biological activity of the ligand. In some cases amine coupling may primarily involve groups that are near or a part of the active site or binding site of the ligand, which may result in inactivity of the ligand. In these cases the ligand can be attached using a capturing coupling method.

Coupling Procedure

1. Surface Conditioning

Perform an injection of 10 mM HCl (pH 2) 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. Ligand Immobilization

Dilute the ligand to be immobilized in the Activation Buffer (or immobilization buffer - see pre-concentration of ligand) 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 EDC/NHS activation to the signal after the ligand immobilization step. In the example shown in Figure 2, it is approximately 650 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.

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

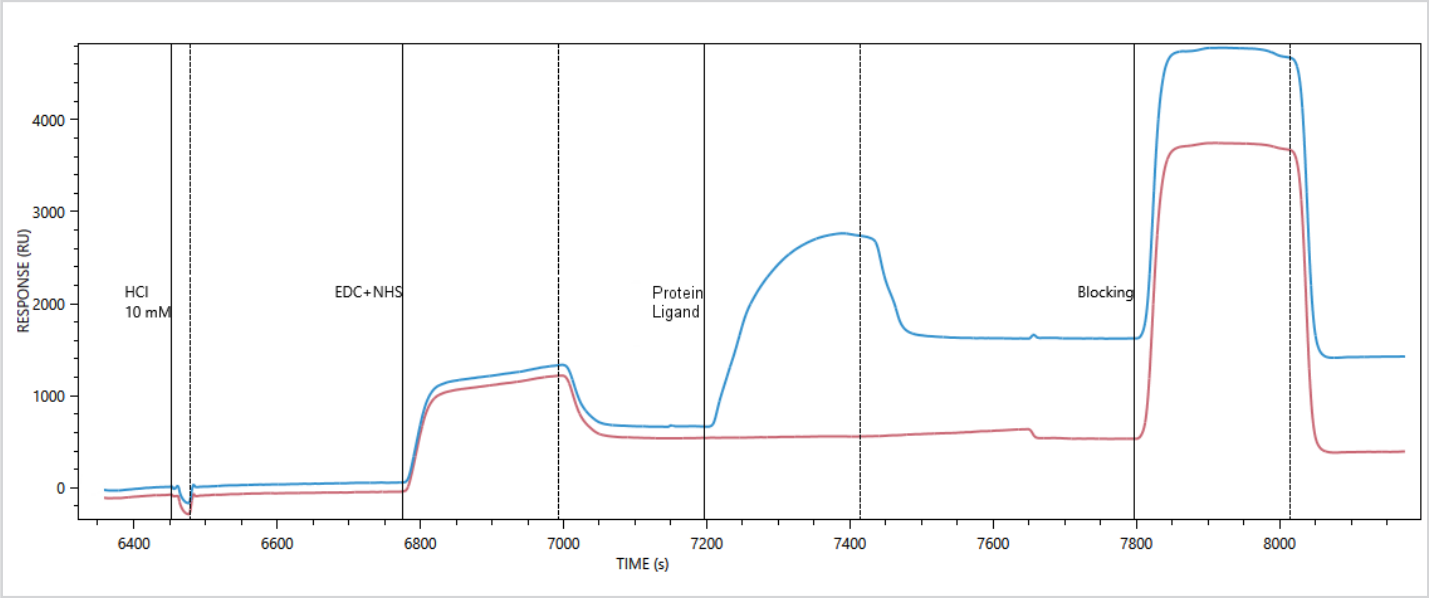

Figure 2. Example of Carboxyl Sensor protein immobilization on the OpenSPR 2-channel system (red: Channel 1, blue: Channel 2).