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XT Tech Guide: COOH Sensors & Amine Coupling Kit for Small Molecule Immobilization

Overview

COOH sensor chips have a uniform layer of carboxyl groups on the surface. These carboxyl groups can be activated with EDC/NHS chemistry to chemically couple the ligand via its amine groups (Figure 1). Nicoya's Amine Coupling Kit contains all reagents necessary to perform this coupling and has been optimized for best performance for small molecule ligands. This coupling method results in stable attachment of the ligand to the surface such that binding interactions can be easily measured with the OpenSPR-XT instrument.

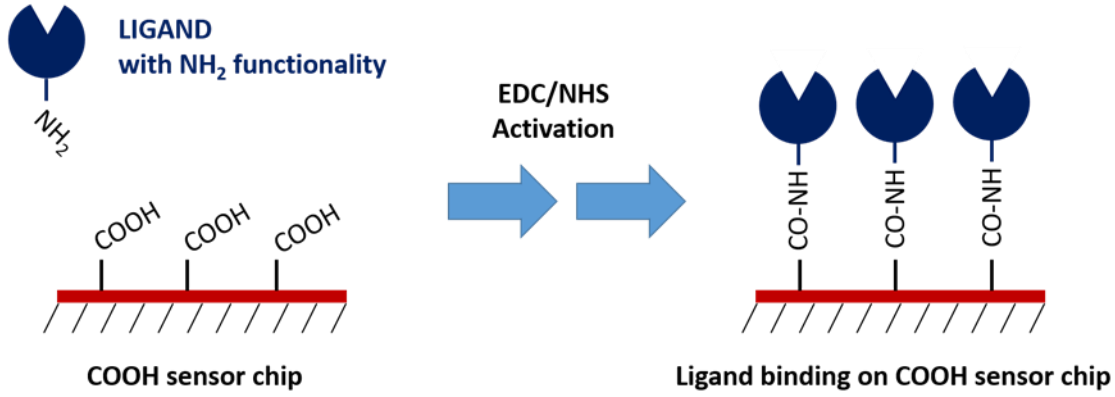

Figure 1. Covalent coupling of ligand to COOH sensor chip.

Materials and Storage Conditions

COOH sensor chips: Store at 2-8°C.
NHS: Store at 2-8°C. Once aliquoted, store at –20°C.
EDC: Store at 2-8°C. Once aliquoted, store at –20°C.
Activation buffer: Store at 2-8°C.
Immobilization buffer: Store at 2-8°C.
Blocking solution: Store at 2-8°C.
10 mM HCl, pH 2 (not provided)

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Preparation of EDC Aliquots

1) Dissolve provided EDC into 1.5 mL activation buffer
2) Pipette 10 aliquots of 150 µL and store at –20°C.

Preparation of NHS Aliquots

1) Dissolve provided NHS into 1.5 mL activation buffer
2) Pipette 10 aliquots of 150 µL and store at –20°C.

Note: Reagents must be prepared immediately before use from aliquots and mixed shortly before use. The EDC/NHS ester has a short half-life, so ligand is be injected shortly after activation. Also, the ligand should be diluted into the immobilization buffer immediately before injection to maintain stability.

Buffer conditions

The immobilization buffer is optimized for small molecules to achieve maximum coupling efficiency with the COOH surface.

Running buffers with primary amine groups or strong nucleophiles like Tris or sodium azide must be avoided for amine coupling as these will compete with the ligand. The running buffer should also not contain BSA for optimal ligand immobilization onto a COOH sensor chip. To reduce non-specific binding of the analyte, BSA can be added into the running buffer after the ligand immobilization steps.

If sufficient immobilization levels cannot be reached with the Amine Coupling Kit, try to increase the ligand concentration.

For more details, refer to the OpenSPR Kinetics Handbook or contact support@nicoyalife.com

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XT COOH Sensor Chip Coupling Procedure: Protein Ligands

Note – this procedure is assuming the use of a 100 µL sample loop in the instrument. Volumes will need to be adjusted if a larger sample loop is installed.

1) Follow the test setup prompts, including priming the system with running buffer and taking new references. When prompted, load a COOH Sensor Chip into the OpenSPR, following the standard procedure.
2) Fill a well with 300 µL 80% isopropanol (v/v in water), and perform a bubble removal pulse injection with this solution. When all bubbles are removed, proceed to the Test Setup-Sample Details screen.
3) Prepare the following samples (excluding those marked by *) to a volume of 300 µL and input the sample information to the corresponding well positions on the Sample Details Screen:
    i) 10 mM HCl (pH 2)
    ii) EDC/NHS*
    iii) Ligand*
    iv) Blocking solution
    v) Regeneration solutions
    vi) Analyte solutions

    * These solutions should only be prepared immediately before the test is commenced
4) On the Test Setup-Test Timeline screen, click the arrow in the top left corner to open the drop down menu and select Open > Template. Select the COOH Sensor Chip template to load it into the test timeline. This will pre-load the recommended test timeline for a COOH sensor chip.
5) Associate the corresponding sample information with the process steps in the Test Timeline and make any necessary customizations.
6) When you are ready to begin your test, dilute the ligand to be immobilized in the immobilization buffer to a volume of 300 µL at a concentration around 1 mg/mL and place it in the corresponding well position indicated in the Sample Details.

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7) Thaw and immediately mix 1 aliquot of EDC and NHS together and place it into the corresponding well position indicated in the Sample Details.
8) Place the 96-well plate(s) into the autosampler chamber and click "Next" to begin your test.
9) You can measure the amount of ligand binding by comparing the signal before EDC/NHS activation to the signal after the blocking step is complete. In the example shown in Figure 2, it is approximately 700 pm. Note this example is for protein immobilization; small molecules will have a much lower signal due to their smaller size.
10) If the optimal regeneration condition is known, it is recommended to perform an injection of the regeneration solution to precondition the ligand at a pump speed of 150 µl/min, as seen in Figure 2 (this is built into the template).
11) Your test will now proceed to your analyte injections.

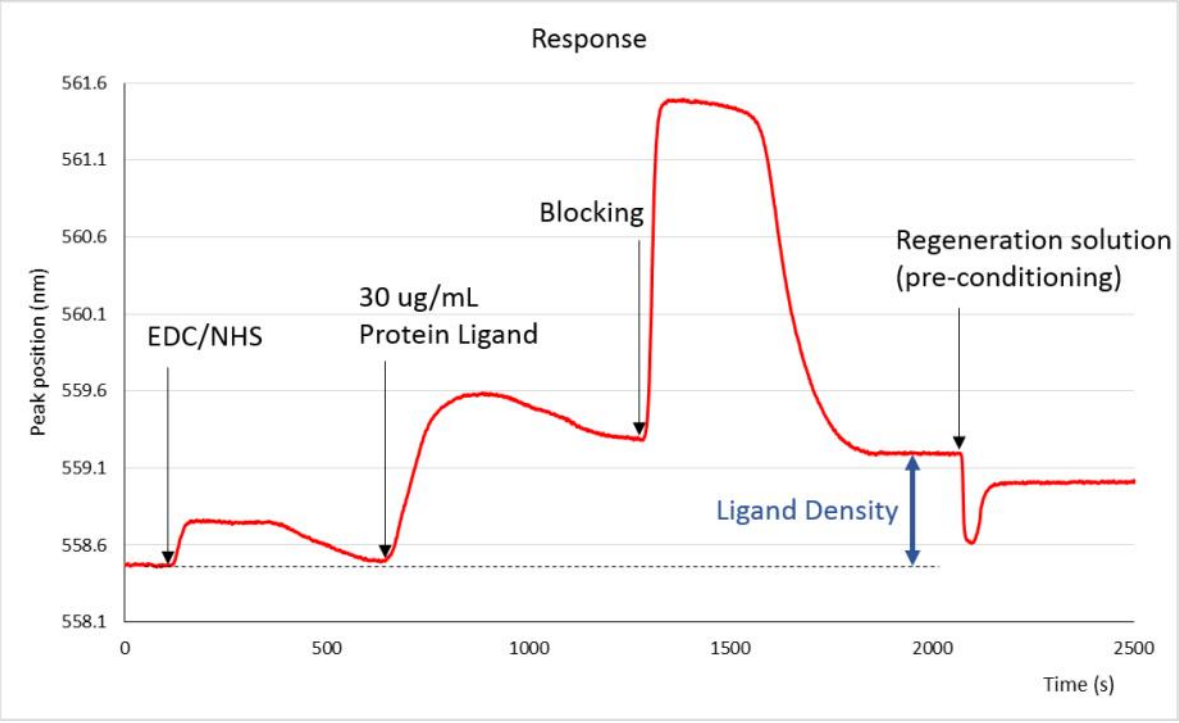

Fig 2. Example of COOH Sensor Chip protein ligand immobilization. Note the expected signal for the ligand density of a small molecule would be at least 10x lower.