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XT Tech Guide: COOH Sensors & Amine Coupling Kit for Protein Immobilization (Sensor Starter Pack)

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 protein ligands. This coupling method results in stable attachment of the ligand to the surface such that binding interactions can be easily measured in the OpenSPR instrument.

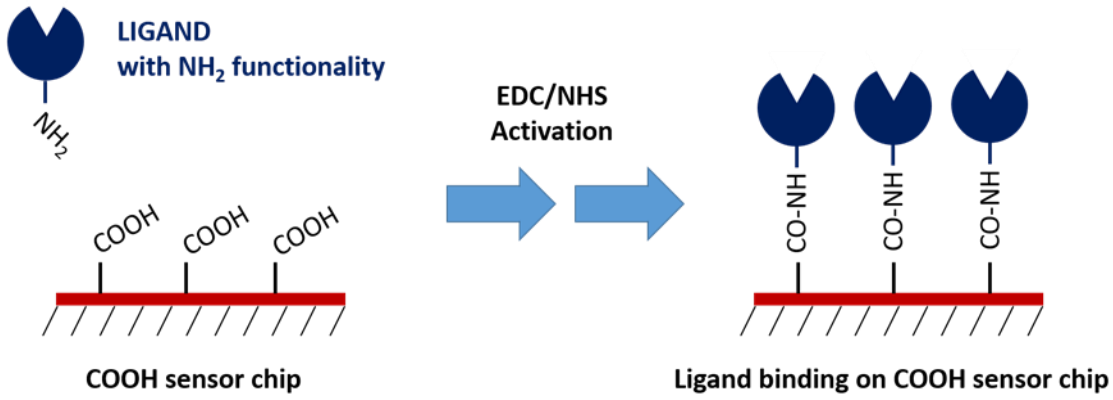

Figure 1. Covalent coupling of ligand to COOH sensor chip.

Materials and Storage Conditions

COOH sensor chips: Stored in PBS buffer. 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.
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 450 µL activation buffer
2) Pipette 3 aliquots of 150 µL and store at –20°C.

Preparation of NHS Aliquots

1) Dissolve provided NHS into 450 µL activation buffer
2) Pipette 3 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 should be injected shortly after activation. Also, proteins are generally not stable for long periods of time in low pH, so ligand should be diluted into the activation buffer and injected immediately.

Buffer conditions

Large ligands such as proteins are usually immobilized to COOH sensor chips using a method known as preconcentration. Preconcentration is the technique in which electrostatic forces are used to increase the local concentration of ligand at the surface of the sensor. Nicoya provides an optimized activation buffer with their Amine Coupling Kit for preconcentration. Ligands that cannot be used for preconcentration should be immobilized through other methods such as capture coupling.

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 activation buffer to a volume of 300 µL at a concentration of 10-50 µg/mL and place it in the corresponding well position indicated in the Sample Details.
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.

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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.
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.

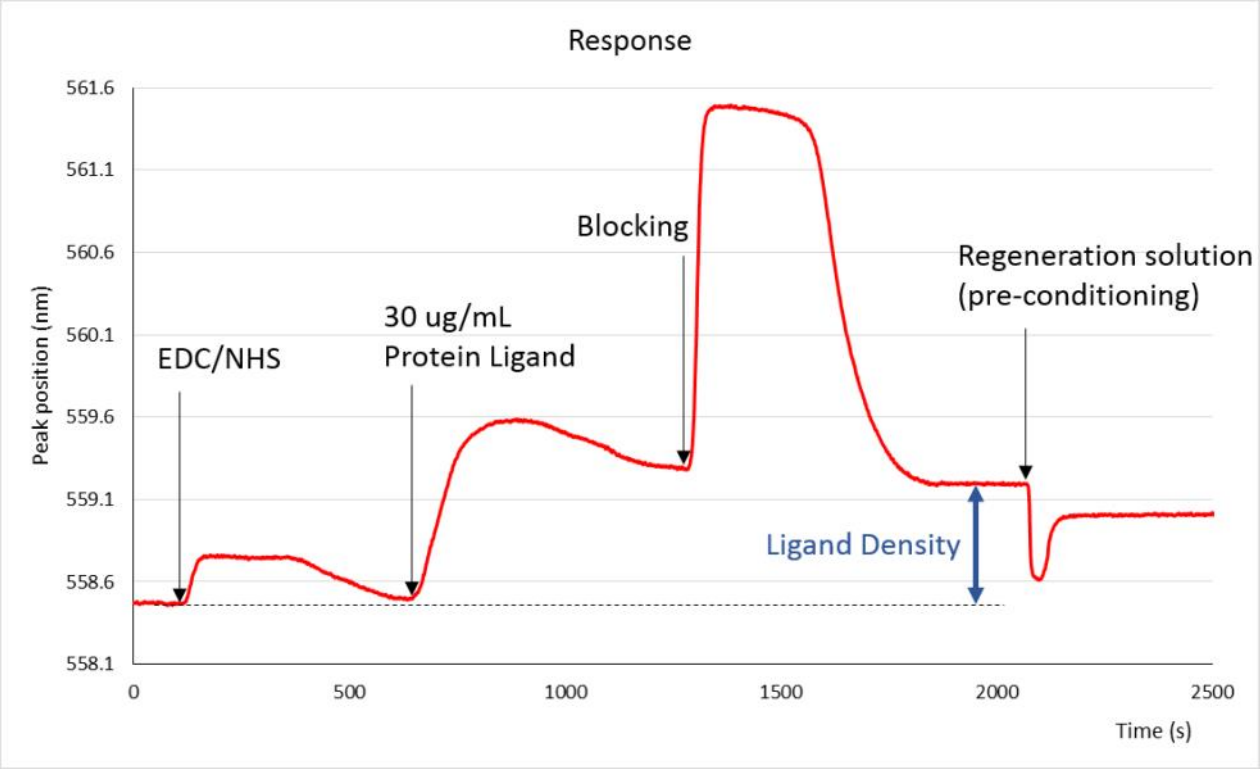

Fig 2. Example of COOH Sensor Chip protein ligand immobilization