



# Carboxyl Reagent & Optimization Kits

<b>Relevant products:</b>	Carboxyl Reagent Kit (COOH-RK-10, COOH-RK-3) Carboxyl Optimization Kit (COOH-OPT-RK-10, COOH-OPT-RK-3) Sensor Starter Pack (SSP-1A)
<b>For use with:</b>	Carboxyl Sensors, High Capacity Carboxyl Sensors, High Sensitivity Carboxyl Sensors

## Overview

The OpenSPR **Carboxyl Reagent Kit** and **Carboxyl Optimization Kit** include all necessary reagents for preparing, activating and blocking the -COOH groups on the surface of OpenSPR Carboxyl Sensors for immobilization of ligands via amine coupling.

When using this coupling method, the EDC/NHS mixture will activate the carboxyl groups on the sensor surface allowing the ligand to be covalently linked via any of its primary amine groups. It is recommended to dilute the ligand into the appropriate immobilization buffer with the optimal pH for pre-concentration (typically 0.5 pH units below the isoelectric point of the protein, however is recommended to test experimentally). Sodium acetate immobilization buffers are provided in the Carboxyl Optimization kit or are available for purchase individually from Nicoya. Once the ligand is coupled to the surface, ethanolamine is then used to deactivate and block the remaining active carboxyl groups on the sensor surface. This method results in stable covalent attachment of the ligand to the sensor surface such that binding interactions can be easily measured with the OpenSPR instrument.

## Kit Contents

Reagent	COOH-RK-10 (10+ reactions)	COOH-RK-3 (3 reactions)	COOH-OPT-RK-10 (10+ reactions)	COOH-OPT-RK-3/ SSP-1A (3 reactions)	Storage upon receipt
10 mM HCl	2x tubes of 1.5 mL	1x tube of 1.5 mL	2x tubes of 1.5 mL	1x tube of 1.5 mL	+4°C
NHS	3x tubes of 4.6 mg	1x tube of 4.6 mg	3x tubes of 4.6 mg	1x tube of 4.6 mg	+4°C
EDC	3x tubes of 7.7 mg	1x tube of 7.7 mg	3x tubes of 7.7 mg	1x tube of 7.7 mg	-20°C
1 M Ethanolamine, pH 8.5	2x tubes of 1.5 mL	1x tube of 1.5 mL	2x tubes of 1.5 mL	1x tubes of 1.5 mL	+4°C
10 mM Sodium Acetate	--	--	1x tube of 2 mL pH 4.0 1x tube of 2 mL pH 4.5 1x tube of 2 mL pH 5.0 1x tube of 2 mL pH 5.5	1x tube of 2 mL pH 4.0 1x tube of 2 mL pH 4.5 1x tube of 2 mL pH 5.0 1x tube of 2 mL pH 5.5	+4°C

# Kit Preparation Procedures

## EDC & NHS Preparation

Unopened tubes of EDC & NHS are recommended to be stored within the resealable bags provided with desiccant to avoid the introduction of moisture.

It is recommended to only prepare 1x tube each of EDC and NHS aliquots at a time, as the shelf-life once dissolved is only 2 weeks. Each tube will make 4x aliquots of each reagent.

### Other materials required

Type I/II water  
Tubes for aliquots (100  $\mu$ L or greater volume)

### Preparation of 0.1 M EDC aliquots

1. Bring 1x tube of EDC powder to room temperature.
2. Add 400  $\mu$ L Type I/II water to the tube of EDC, mix or vortex to dissolve.
3. Pipette 4 aliquots of 100  $\mu$ L each.
4. If not using aliquots immediately, store at  $-20^{\circ}\text{C}$  for up to 2 weeks.

### Preparation of 0.1 M NHS aliquots

1. Bring the tube of NHS powder to room temperature.
2. Add 400  $\mu$ L Type I/II water to the tube of NHS, mix or vortex to dissolve.
3. Pipette 4 aliquots of 100  $\mu$ L each.
4. If not using aliquots immediately, store at  $-20^{\circ}\text{C}$  for up to 2 weeks.

Immediately before use in the experiment, one 100  $\mu$ L aliquot of EDC and one 100  $\mu$ L aliquot of NHS shall be mixed together, creating a final concentration of 0.05 M each at a 1:1 ratio. The EDC/NHS ester has a short half-life, so the ligand should be injected shortly after activation. See Sensor Chip Tech Guide for experiment procedure details.

**!! Note the shelf life of prepared aliquots is 2 weeks from the date of preparation.** It is not recommended to use the aliquots after 2 weeks as it can result in reduced activity of the reagents and lower ligand immobilization.

## Additional Notes

10 mM HCl and 1 M Ethanolamine solutions come ready to use and require no additional preparation.

The ligand will be diluted into a sodium acetate immobilization buffer at a recommended concentration of 10-50  $\mu\text{g}/\text{mL}$  immediately before use in the experiment.

The Carboxyl Optimization Reagent Kit provides immobilization buffers from pH 4.0-5.5 for pre-concentration of most proteins to the carboxyl sensor surface. In theory, the optimal buffer pH for pre-concentration is typically 0.5 pH units below the isoelectric point of the protein, however it is recommended to perform a pre-concentration screen of the ligand to determine the optimal immobilization buffer pH experimentally. The buffer condition that enables the highest pre-concentration at the highest pH should be selected for use. Ligands that cannot be used for pre-concentration are recommended to be immobilized through other methods such as capture coupling (streptavidin-biotin or His tag-NTA), or may need to be used at a higher concentration to effectively couple to the carboxyl surface.