

NHS COUPLING KIT

Code Number NHS-2005

Contents N-Hydroxysuccinimide (NHS) {**0.2 grams}
1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) {**1.15 grams}
1M Ethanolamine, pH 8.5 (Deactivation Buffer){**25ml}

{**For information only, weights and volumes quoted do not conform to any standards.}

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STORAGE & SHELF LIFE

*** DISSOLVE AND ALIQUOT NHS & EDC IMMEDIATELY ***

Once the EDC and NHS have been reconstituted in high purity water the solutions must be stored below -18°C and used within 6 months from the date of reconstitution. Ethanolamine should be kept in the dark at room temperature, and under these conditions is stable for 6 months. Kits should not be reconstituted after the expiry date shown on the box. See 'PREPARATION OF REAGENTS' below.

PREPARATION OF REAGENTS

URGENT: For best results dissolve the reagents immediately upon receipt and store below -18°C. Dissolve the EDC in 15 ml of freshly drawn high purity water and mix until completely dissolved. Aliquot into 250 µl volumes and store below -18°C. Dissolve the NHS in 15 ml of freshly drawn high purity water and mix until completely dissolved. Aliquot into 250 µl volumes and store below -18°C. It is recommended that to avoid confusion that the reagents are made up one at a time and aliquoted fully before starting the next. A good idea is to use two different coloured microcentrifuge tubes, enabling easy identification of the two reagents. For use in immobilisation protocols one vial of each, NHS and EDC, should be thawed just prior to use. Mixing together of an equal volume of each reagent results in an activation solution of the recommended strength (0.2 M EDC and 0.05 M NHS).

PRODUCT DESCRIPTION

This product is for use in covalent immobilisation of amine containing ligands to the carboxyl groups on the IAsys CM-Dextran, CM-Dextran select, and Carboxylate cuvettes. Full details of the methodologies involved can be found in **Protocol notes 1.1** (CM-Dextran) and **1.3** (Carboxylate). Copies can be obtained by e-mail from iasys.support@thermobio.com, or from the web www.affinity-sensors.com.

Figure 1 below shows a schematic of the coupling chemistry for the CM-Dextran cuvettes, however the basic chemistry applies to the Carboxylate cuvettes. Briefly the carboxyl groups

are activated to NHS esters, followed by displacement of the NHS by amine groups on the ligand. This results in the covalent attachment of the ligand via peptide bonds.

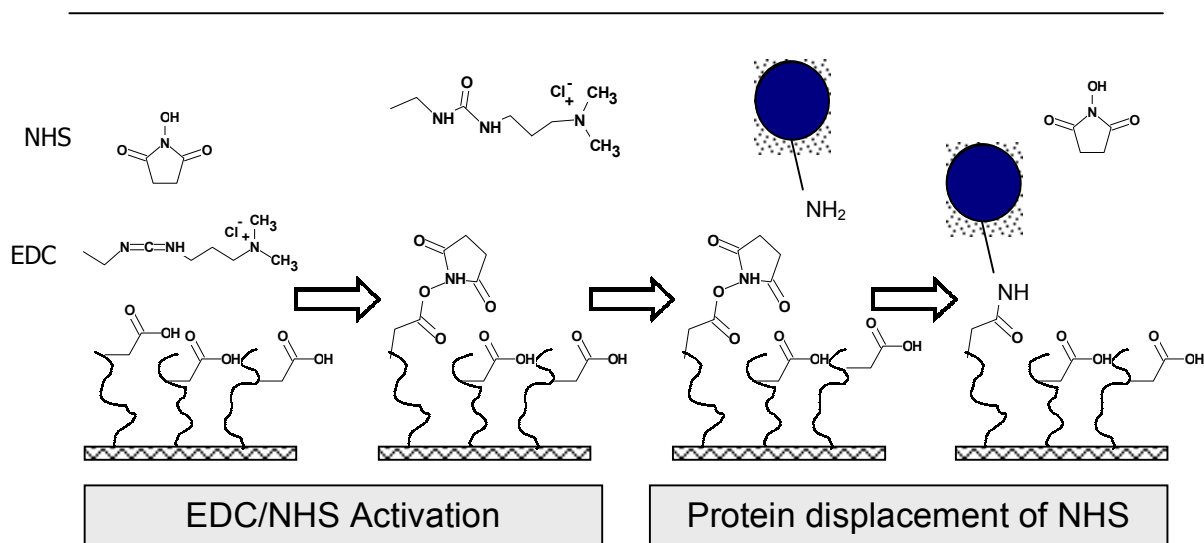


Figure 1 Reaction scheme for EDC/NHS mediated amine coupling to a CM Dextran cuvette.

Other nucleophilic groups such as phenolic groups in tyrosine and imidazole groups in histidines may also be involved to a lesser extent. For this reason it is important to avoid azide and TRIS in the ligand buffer.

Activated groups to which ligand has not been coupled can be deactivated using the ethanolamine supplied.

The descriptions contained herein were correct at the time of printing and are subject to alteration to alteration without notice. Users and managers are reminded that they share a responsibility for controlling any risk to the environment and for safeguarding the health and safety of persons and property. The goods especially chemicals, should be properly stored, used and disposed off at all times in accordance with safe working routines and only used by competent persons.

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