SARTORIUS

Octet® Amine Reactive Second-Generation (AR2G) Reagent Kit

Technical Note

Scope
This technical note provides guidance for AR2G immobilization and recommendations for streamlining assay development.

Abstract

Amine coupling chemistry is the most widely used covalent immobilization method. Because most proteins contain multiple amine groups, immobilization of the protein is easily achieved without denaturation and inactivation of the protein. The Octet® Amine Reactive 2nd Generation (AR2G) Reagent Kit enables covalent immobilization of a protein, peptide or other primary amine-containing biomolecule onto the AR2G Biosensor surface via a stable amide bond. This technical note provides guidance for immobilization and recommendations for streamlining assay development.

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Overview

The Octet® Amine Reactive 2nd Generation (AR2G) Reagent Kit is intended for use with the AR2G Biosensor to enable covalent immobilization of a protein, peptide or other primary amine containing biomolecule onto the AR2G Biosensor surface via a stable amide bond. Immobilization of proteins is achieved through standard EDC-catalyzed amide bond formation to create a covalent bond between a reactive amine on the protein and the carboxy-terminated biosensor surface. The kit contains sufficient reagents to perform 1000 standard immobilization reactions.

Principle

The Octet® Amine Reactive 2nd Generation (AR2G) Reagent Kit includes all of the coupling, quenching and assay buffers needed to immobilize proteins onto AR2G Biosensors. The kit includes both EDC (1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride) and s-NHS (N-hydroxysulfosuccinimide), which react with the carboxy-terminated AR2G Biosensor to produce sulfo-NHS esters. The esters rapidly react with the primary amines of proteins, peptides and other biomolecules to form highly stable amide bonds. To streamline assay development, stock solutions of 10 mM acetate at three different pHs are included in the kit to facilitate optimizing the immobilization conditions. For most biomolecules, the acetate buffer generates robust and high quality immobilization results. However, alternative non-amine buffers, such as MES, may be evaluated for further optimization. Excess sulfo-NHS esters are quenched using 1M ethanolamine. The resulting immobilized peptide or protein biosensor can be used for screening or kinetic characterization experiments. Sartorius' standard 10X Kinetics Buffer and PBS are also included in the kit.

Materials Included

- EDC (1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride), 1015 mg
- S-NHS (N-hydroxysulfosuccinimide), 573 mg
- 1M ethanolamine pH 8.5, 200 mL
- 10 mM Acetate pH 4.0, 200 mL
- 10 mM Acetate pH 5.0, 200 mL
- 10 mM Acetate pH 6.0, 200 mL
- PBS pH 7.4, 500 mL
- 10X Kinetics Buffer, 50 mL

Additional materials required

- Nanopure water

Tips for Optimal Performance

- Store reagents at the temperatures indicated on each bottle label. Reagents have different storage conditions.
- When ready to use, dissolve the EDC and s-NHS in water as described, aliquot into tubes and freeze. Aliquots of 100 µL will provide a sufficient amount of reagent to perform 16 immobilizations in a 96-well plate at the standard EDC concentration. Store at –20°C until needed. Reagent integrity will be maintained for at least 6 months under proper storage conditions.
- For each experiment, thaw 1 aliquot of EDC and 1 of s-NHS. Vortex briefly after thawing.
- Use or refreeze EDC and s-NHS aliquots within 10 hours of thawing.
- Use EDC and s-NHS within 1 hour of mixing them together.

Reagent Preparation

Overview

1. Aliquot and freeze EDC and s-NHS stocks
2. Prepare 1X Kinetics Buffer

Aliquot and Freeze EDC and s-NHS Stocks

For long-term storage (over 6 months) it is best to store the EDC and s-NHS in powder form at the temperatures indicated on the labels.

For short-term use and ease of use, it is recommended to dissolve each reagent in water and prepare working size aliquots for storage at –20°C.

1. Prepare EDC aliquots.
   a. Dissolve EDC in 13.2 mL of nanopure water to prepare a 400 mM stock. Mix to ensure complete dissolution of the solid.
   b. Aliquot into labeled microfuge tubes. Aliquots of 100 µL will provide a sufficient amount of reagent to perform 8 immobilizations in a 96-well plate at the standard EDC concentration.
   c. Freeze immediately at –20°C. Store at –20°C until needed.
2. Prepare s-NHS aliquots.
   a. Dissolve s-NHS in 13.2 mL of nanopure water to generate a 200 mM stock. Mix to ensure complete dissolution of the solid.
   b. Aliquot into labeled microfuge tubes. Aliquots of 100 µL will provide a sufficient amount of reagent to perform 8 immobilizations in a 96-well plate at the standard s-NHS concentration.
   c. Freeze immediately at –20°C. Store at –20°C until needed.

Prepare 1X Kinetics Buffer

The AR2G Biosensor is compatible with a wide variety of buffers (HBS, PBS, etc) for kinetic assays. For convenience, Sartorius' Kinetics Buffer is included within the AR2G reagent kit. Kinetics Buffer is PBS based (pH 7.4) and includes both carrier protein (BSA) and a dilute concentration of detergent.

1. Dilute the 10X Kinetics Buffer stock with the provided PBS 1:10.
2. Store at room temperature until needed. For long term storage, store 1X Kinetics Buffer at 4°C.

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