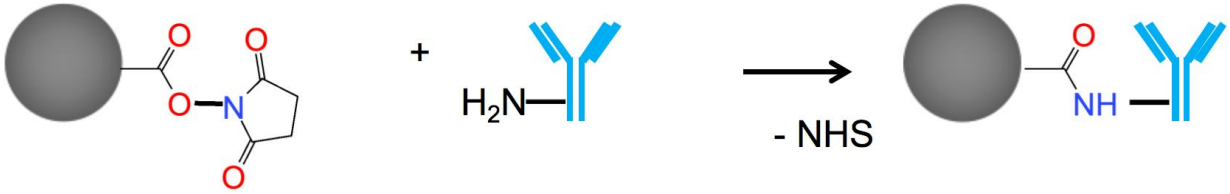
Coating of Carboxyl Particles with Proteins or Ligands

Introduction

There are several methods of attaching biological ligands to polystyrene particles. These methods include adsorption to plain polystyrene particles, covalent attachment to surface functionalized particles, and attachment of the ligand of interest to particles that are pre-coated with a binding protein such as Streptavidin, Protein A or Protein G.

An easy and rapid conjugation method of biomolecules to particle surfaces is based on the carbodiimide / N-hydroxysuccinimide activation of the carboxylic acid groups on particle surfaces followed by reaction with amino groups of the biomolecule.

Presented in this Technical Note are protocols such as a random amide bond formation between particle surface and biomolecule, and is limited regarding the oriented binding of molecules, like antibodies, on the particle surface.



Material

Carboxyl Particles

0.5 M MES, pH = 6.3

EDC

NHS

50-100 µg protein or antibody per 10 mg Carboxyl Particles

0.01 M PBS, pH = 7.4

Glycine in 0.01 M PBS buffer

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Procedure

1. Transfer the particle suspension into a 2 ml reaction tube.
2. Dissolve EDC and NHS in 0.5 M MES (pH = 6.3).
3. Incubate the suspension with continuous shaking at room temperature.
4. Wash the activated particles with 0.01 M PBS (pH=7.4).
5. Add 0.01 M PBS (pH = 7.4) containing protein or antibody.
6. Incubate the suspension with continuous mixing at room temperature.
7. Wash the particles with 0.01 M PBS (pH=7.4).
8. Add glycine in 0.01 M PBS.
9. Incubate the suspension with continuous mixing at room temperature.
10. Wash the particles three times with 0.01 M PBS (pH=7.4).
11. Resuspend the particles in PBS (pH = 7.4).
12. Stabilize the suspension by addition of 20 µl 1% sodium azide solution if necessary.

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