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TECHNOTE 200

Binding of Biomolecules on the Surface of Carboxylated Particles by Carbodiimide Chemistry

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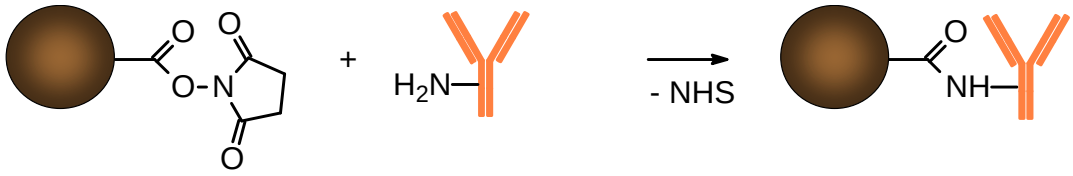
Introduction

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 [1]-[6]:



This method leads to 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 [7].

Protocol

The protocol is given for the coating of a fixed amount of 25 mg of particles with a special protein or antibody. It can be varied in the scale according to your individual requirements.

Material:
- particle suspension (surface: COOH or PEG-COOH) containing 25 mg of particles,
- 0.5 M MES buffer (2-(4-morpholino)ethanesulphonic acid buffer), which was adjusted to pH 6.3 with 2.5 M Na_2CO_3,
- 4 mg EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride),
- 8 mg NHS (N-hydroxysuccinimide),
- 150 – 200 µg protein or antibody,
- 0.01 M PBS buffer (pH = 7.4),
- 200 µl of 25 mM glycine in 0.01 M PBS buffer.

Procedure:
- transfer the particle suspension into a 2 ml reaction tube,
- dissolve 4 mg EDC and 8 mg NHS in 0.5 M MES-buffer (pH = 6.3). The buffer volume should be a quarter of the initial volume of the particle suspension.
- incubate the suspension with continuous shaking for 45 min at room temperature,
- wash the activated particles by centrifugation (Technote 100), magnetic separation (Technote 101) or size exclusion chromatography (Technote 102) with 0.01 M PBS buffer (pH=7.4),
- add 0.01 M PBS buffer (pH = 7.4) containing 150 – 200 µg protein or antibody,
- incubate the suspension with continuous mixing for 3 hours at room temperature,

- wash the particles by centrifugation (Technote 100), magnetic separation (Technote 101) or size exclusion chromatography (Technote 102) with 0.01 M PBS buffer (pH=7.4),
- add 200 µl of 25 mM glycine in 0.01 M PBS buffer,
- incubate the suspension with continuous mixing for 30 min at room temperature,
- wash the particles three times by centrifugation (Technote 100), magnetic separation (Technote 101), size exclusion chromatography (Technote 102) or dialysis (Technote 103) with 0.01 M PBS buffer (pH=7.4),
- resuspend the particles in 1 ml PBS-buffer (pH = 7.4),
- stabilize the suspension by addition of 20 µl 1% sodium azide solution if necessary.

Note:
This protocol is intended to provide general guidelines for the binding of biomolecules or related compounds. Further optimization may be required in order to achieve optimal functionality and stability from case to case.

References

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