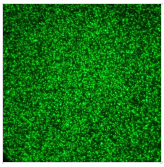

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Covalent Coupling Protein to Carboxylated Microparticles via EDAC

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bhsvj6e6

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ABSTRACT

This protocol details how to covalently couple proteins to carboxylated polystyrene particles (microspheres) via EDAC. In this reaction, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) serves as an agent for covalently coupling protein (e.g. peptides, IgG, etc.) to carboxyl-modified particles by forming an amide bond. Various 2-(*N*-morpholino)ethanesulfonic (MES) acid buffers are used to maintain the appropriate pH ranges. Glycine is used as a quencher to cease the reaction. Bovine serum albumin (BSA) is used as a blocking agent, to prevent non-specific interactions with and between particles (e.g. hydrophobic interactions).

This protocol's development was guided by the information contained in Bangs Laboraties Inc. Tech Note 205, Covalent Coupling. This file is attached as a PDF.

ATTACHMENTS

bangs_laboratories_technote205_covalent_coupling.pdf

DOI

dx.doi.org/10.17504/protocols.io.bhsvj6e6

PROTOCOL CITATION

Kenneth Schackart, Kattika Kaarj 2021. Covalent Coupling Protein to Carboxylated Microparticles via EDAC . **protocols.io** https://dx.doi.org/10.17504/protocols.io.bhsvj6e6

KEYWORDS

covalent coupling, carbodiimide, carboxylated polystyrene, polystyrene microspheres, EDC chemistry, peptide conjugation, anitbody conjugation, protein conjugation, microparticles

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38453

GUIDELINES

When using fluorescent particles, avoid prolonged exposure to light as this may cause photobleaching.

MATERIALS TEXT

Materials

- 2 mL centrifuge tubes & tube holder
- Aluminum foil
- Carboxylated polystyrene particles (this protocol is based on 0.5 µm diameter, **2.5 % w/v** stock)
- Microcentrifuge
- Orbital mixer
- Vortex mixer
- Sonication bath

Reagents

- Activation buffer
- Coupling buffer
- Quenching solution
- Blocking solution (optional)
- Storage buffer
- 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) **0.1 % w/v**

Recommended concentrations from Bangs Laboratories Inc. TechNote 205 Covalent Coupling:
Buffer:

- **19.2 g** MES free acid (MW = **195.2 g/mol**) in ~ **900 mL** deionized water (DW)
- Adjust pH with **1 Molarity (M)** HCl or **1 Molarity (M)** NaOH
- Adjust to final volume (~ **1000 mL**) with DW
- Activation buffer: pH **4.5** - pH **7.5**
- Coupling buffer: pH **7.2** - pH **8.5**

Quenching solution:

- **30 Milimolar (mM)** - **40 Milimolar (mM)** Glycine in **0.05 % w/v** - **1 % w/v** BSA

Activation buffer (MES: 100 Milimolar (mM) , pH 6.0)

- **4.8 g** MES in **225 mL** DW (prepare in 500 mL flask)
- Adjust to pH **6.0**

EDAC - 0.1 % w/v (prepare fresh for each conjugation)

- Add **5 mL** Activation buffer to **5 mg** pre-aliquoted EDAC (in 5 mL balck tube, stored in deep freezer)

Coupling buffer (MES: 100 Milimolar (mM) , pH 8.0)

- **4.8 g** MES in **225 mL** DW (prepare in 500 mL flask)
- Adjust to pH **8.0**

Quenching solution (35 Milimolar (mM) Glycine and 0.1 % w/v BSA)

- Add **100 mg** bovine serum albumin (BSA) in **100 mL** DW

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- Add **263 mg** Glycine to BSA solution

Blocking solution ([M]1 % w/v BSA in DW)

- Add **1 g** BSA in **100 mL** DW

Storage buffer (MES: [M]100 Milimolar (mM) , pH7.0 and [M]0.1 % w/v BSA)

- **4.8 g** MES in **225 mL** DW (prepare in 500 mL flask)
- Adjust to **pH7.0**
- Add **250 mg** BSA (in **250 mL** MES solution)

BEFORE STARTING

Ensure that all buffers are prepared. Take EDAC aliquot from deep freezer, and allow to reach room temperature.

Wash Particles 1h

1 Sonicate carboxylated polystyrene particle stock in sonicating bath for **00:10:00** .

Vortex mix particle stock for **00:00:30** .

2 Add **10 µl** particles to 2 mL centrifuge tube.

Add **500 µl** Activation buffer.

3 Vortex particle mixture.

Centrifuge at **9900 x g, 00:10:00**

Remove supernatant.

Resuspend in **500 µl** Activation buffer.

Repeat for a total of two washes; *Do not resuspend in Activation Buffer the second time.*

All centrifugation times and speeds will depend on particle size. They will need to be adjusted accordingly.

Bind EDAC to Particles 25m

4 During the centrifugation times in Step 3, make EDAC solution:

- Add **5 mL** Activation buffer to **5 mg** EDAC aliquot.
- Vortex mix.

This amount of EDAC is in excess. Tech Note 205 uses solution with100 mg EDAC for 100 mg particles. The excess EDAC is mostly removed by centrifugation.

5 Resuspend particle pellet from Step 3 with EDAC/Activation buffer, ensuring that particles are well suspended.

Agitate on orbital mixer, **00:15:00** .

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6 Vortex particle mixture.
Centrifuge at **9900 x g, 00:10:00** .
Remove supernatant.
Resuspend in Coupling buffer.
Repeat for a total of two washes.

Couple Protein to Particle 3h 30m

7 During the centrifugation times in Step 6, make Protein solution:
- Add protein for 1-10X coverage (as calculated below) to **500 µl** Coupling buffer.
- Vortex mix.

The amount of protein (e.g. antibody, peptide) will depend on particle concentration, size, etc. The following equation is used to calculate the necessary amount (adapted from TechNote 205):

$$S = \frac{6C}{\rho d}$$

- S (mg protein / g particles): amount of protein for surface saturation
- C (mg protein / m^2 particle surface): capacity of particle surface for a given protein
- ρ (g / cm^3): density of particle
- d (µm): mean particle diameter.

8 Combine pellet suspension and Protein solution.
Pipet mix.
Agitate on orbital mixer, **02:00:00** to **04:00:00** at room temperature.

9 Centrifuge at **9900 x g, 00:10:00** .
Remove supernatant.
Resuspend particle pellet with **500 µl** Coupling buffer
Vortex mix.
Centrifuge at **9900 x g, 00:10:00** .

Quench Coupling Reaction 30m

10 Resuspend particle pellet from Step 9 with **1 mL** Quenching solution.
Agitate on orbital mixer, **00:30:00** at room temperature.

Incubate with Blocking Buffer (optional) 25m

11 Centrifuge at **9900 x g, 00:10:00** .
Remove supernatant.
Resuspend particle pellet with **500 µl** Blocking solution
Agitate on orbital mixer, **00:15:00** at room temperature.

Wash and Resuspend 20m

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12 Centrifuge at **9900 x g, 00:10:00** .

Remove supernatant.

Resuspend particle pellet with **500 µl** Storage buffer.

Vortex mix.

13 Centrifuge at **9900 x g, 00:20:00** .

Remove supernatant.

Resuspend particle pellet with Storage buffer to desired storage concentration.

Store at **4 °C** .

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