Protocol

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Coupling Protocol for Latex Carboxyl Modified Particles

Introduction

Agilent has been manufacturing polystyrene latex particles for almost three decades, focusing on two main carboxyl product lines: carboxyl beads (larger parking area and lower surface density) and supercarboxyl beads (lower parking area and larger surface density). Applications like turbidimetry and lateral flow assays will require coupling of a biomolecule to the surface. For successful coupling of biomolecules to latex particles bearing surface carboxylic (COOH) groups, the following protocol should be followed. Agilent strongly recommends further optimization to achieve the best performance in your application.

EDC/S-NHS protein-binding protocol

Material required to perform the coupling protocol

- Reverse osmosis (RO) water
- 0.1 M MES (2-[morpholino]ethanesulfonic acid), pH 5
- EDC (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide) >98% Purum; mol wt 191.7 g/mol
- S-NHS (N-Hydroxysulfosuccinimide).

Note: NHS (N-hydroxyl succinimide) can potentially be used instead of sulfo-NHS. However, for higher efficiency, Agilent strongly recommends using sulfo-NHS as a co-activator.

- Phosphate-buffered saline (PBS): 0.1 M sodium phosphate, pH 7.4
- Protein solution: 0.5 mL of protein prepared in PBS buffer.

Note: Titration of the protein amount is highly recommended. See step 5 and Table 2 for suggested starting values.

- 0.1 M Trizma-HCl for quenching EDC activation reaction
- Blocking buffer: 0.1 M sodium borate (boric acid + NaOH), 0.5% Tween-20, pH 8.7
- 25 mM Glycine pH 8.2 buffer (for long storage periods it is recommended to add 0.1w% of sodium azide to avoid microbial growth)
- Vortex mixer
- Centrifuge
- Bottle roller
- Sonic probe to redisperse the latex and obtain desirable size distribution (Agilent recommended but optional)

Before starting

- Consider the choice of latex beads as they can be critical to performance depending on the application. We recommend testing both carboxyl and supercarboxyl beads to evaluate the best performance in your application.
- Check the expiration date on buffers (6 months from preparation)
- Allow the reagents to reach room temperature (except EDC, which should be taken out from freezer when needed).
- Disperse the beads using a bottle roller for a minimum of 2 hours for volumes over 250 mL, or 1 hour for smaller volumes. Nonhomogeneous dispersion could result in an incorrect quantity of beads being used in conjugation or aggregations, causing incomplete coupling. For optimal bead dispersion, Agilent recommends rolling the beads at room temperature overnight (~16 hours).
- Dialysis and concentration measurement of the protein in PBS buffer should be carried out in advance.

Procedure

1. Aliquot 10 mg (0.1 mL) of latex beads in a 2 mL clear Eppendorf tube.
2. Add 1 mL of PBS buffer to the beads and mix the latex with the PBS buffer using a vortex mixer for 10 seconds. Once the beads have been redispersed, place the tube in a centrifuge at 15,000 rpm to separate the latex from solution (see Table 1 for separation times depending on the latex size). Once the separation has been completed, remove the supernatant with a Pasteur pipette (washing step).

Note: for large volumes, ion exchange resins can be used to perform the initial wash; mix 2 mL of latex beads (200 mg) with 400 mg of ion exchange resins. Roll the beads for 2 hours. Filter the resins using a 30 µm nylon mesh or a 5 µm PVDF filter. Solids content should be measured if this process is applied. This step is advised to be performed the day before the protocol.

Table 1. Spin times for different bead size latex at 15,000 rpm (21,100 RCF) in a standard benchtop centrifuge. Higher rpm can be used to decrease the spin time.

Bead Size (nm)	rpm at Room Temperature	Time (min)
100	15,000	60
200	15,000	20
400	15,000	10

3. Weigh 20 mg of EDC and 10 mg of S-NHS reagents in a 15 mL tube (in this order, at a ratio of 2/1 EDC/S-NHS) and add 10 mL of 0.1 M MES pH 5 buffer (activation solution). Tip: weigh the EDC and S-NHS reagents while the beads are in the centrifuge, but do not add the MES pH 5 buffer until PBS aspiration is completed to avoid EDC hydrolysis.
4. Aspirate the PBS buffer from the latex and immediately add 10 µL of the activation solution, followed by 500 µL of RO water. Mix the beads using the vortex mixer for 20 seconds or, if necessary, sonicate the tube until the beads are redispersed. Roll the beads at room temperature for 20 minutes.

Note: this step should be completed in less than 5 minutes to achieve an optimum activation.

5. Add 500 µL of protein solution (see Table 2 for recommended protein amount, depending on the latex size) to the bead and activators mixture. Roll the beads for 45 minutes at room temperature.

Table 2. Recommended protein amounts for different latex sizes.

Bead Size (nm)	Protein Concentration (mg/mL)	Protein Amount (µg/mg bead)
100	0.8	40
200	0.5	25
400	0.3	15

Note: These EDC/S-NHS amounts and protein concentrations have been optimized based on CRP protein. This is to achieve a maximum binding capacity and avoid polymerization or bead aggregation that can disrupt the assay performance. During this optimization process, it was observed that PBS buffer promotes bead agglomeration and subsequent precipitation of latex beads into the media. To avoid the loss of colloidal stability, it is strongly recommended to avoid PBS-containing buffers after this step. If other proteins are coupled to the beads, a titration of the protein amount should be carried out to achieve optimal assay performance.

6. To deactivate the uncoupled activated carboxyl groups, add 50 µL of Trizma-HCl buffer to the latex mixture and roll the beads for 10 minutes.
7. Centrifuge the tubes for 15 minutes, aspirate the supernatant, and add 1 mL of blocking buffer to the beads.
8. Sonicate the samples using a sonic probe at low amplitude for 30 seconds. If redispersion is not successful, repeat the process until the beads are not aggregated. Avoid sample warming by placing the sample on an ice bath.
9. Roll the beads at room temperature overnight.
10. Centrifuge the tube to separate and aspirate the supernatant.
11. Wash the beads with 1 mL of glycine buffer, as indicated in step 2.
12. Resuspend the beads in 1 mL of glycine buffer and treat the samples thermally at 37 °C for three days.

Note: this time should be optimized.

13. Centrifuge the tube to separate, then aspirate the supernatant and resuspend the beads in 1 mL glycine buffer. Roll the beads for at least 1 hour before using the samples in any assay.
14. Store the beads at 2 to 8 °C. For long-term storage, it is recommended to add 0.1 w% of sodium azide as a preservative to avoid microbial growth.

Parking area definition

Parking area (PA): the space occupied by a single COOH group (see Figure 1).

Surface charge density (SCD): the amount of functional groups present on the surface of the beads (see Figure 1).

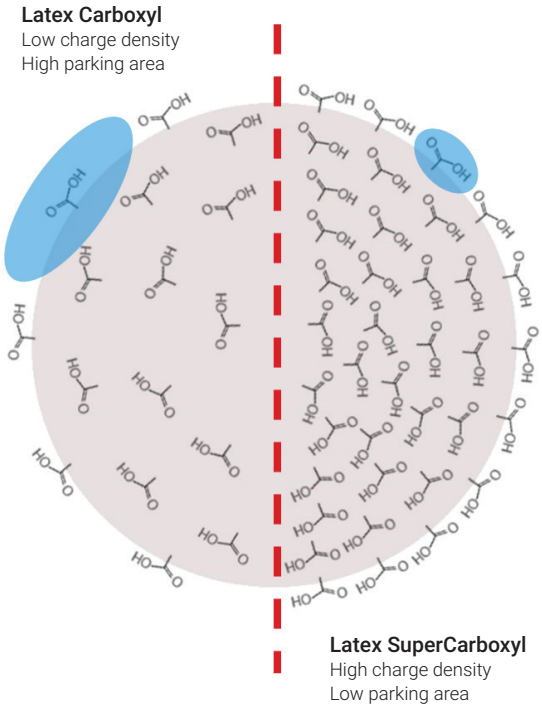

Figure 1. Parking area representation of latex carboxyl and supercarboxyl beads.

Surface charge density (SCD) is usually measured by titrating the COOH groups present in the surface of the bead with a base. Once this measurement is carried out using the diameter (D) and the density (ρ) of the particle, the parking area can be calculated using the following equation (Equation 1):

$$\text{PA (Å}^2\text{/charge group)} = \frac{1}{1.004 \times D\ (\mu m) \times \rho(g/L) \times SCD(meq./g)}$$

Equation 1. Parking area formula.

Ordering Information

Carboxyl-modified products

Latex Carboxyl White beads	15 mL	100 mL	1 L
100 nm 10% Solids	PL6101-6101	PL6101-6102	PL6101-6103
150 nm 10% Solids	PL6115-6101	PL6115-6102	PL6115-6103
200 nm 10% Solids	PL6102-6101	PL6102-6102	PL6102-6103
300 nm 10% Solids	PL6103-6101	PL6103-6102	PL6103-6103
400 nm 10% Solids	PL6104-6101	PL6104-6102	PL6104-6103

Latex SuperCarboxyl White beads	15 mL	100 mL	1 L
50 nm 10% Solids	PL6200-6101	PL6200-6102	PL6200-6103
100 nm 10% Solids	PL6201-6101	PL6201-6102	PL6201-6103
125 nm 10% Solids	PL6212-5101	PL6212-5102	PL6212-5103
150 nm 10% Solids	PL6215-6101	PL6215-6102	PL6215-6103
200 nm 10% Solids	PL6202-6101	PL6202-6102	PL6202-6103
300 nm 10% Solids	PL6203-6101	PL6203-6102	PL6203-6103
400 nm 10% Solids	PL6204-6101	PL6204-6102	PL6204-6103

Latex Carboxyl HiDye beads	15 mL	100 mL	1 L
Blue			
200 nm 10% Solids		Available on request	
300 nm 10% Solids		Available on request	
400 nm 10% Solids		Available on request	
800 nm 10% Solids		Available on request	
Purple			
300 nm 10% Solids		Available on request	
Red			
200 nm 10% Solids		Available on request	
300 nm 10% Solids		Available on request	
400 nm 10% Solids		Available on request	
600 nm 10% Solids		Available on request	
Green			
300 nm 10% Solids		Available on request	
Blue			
400 nm 10% Solids	PL6104-6121	PL6104-6122	
Red			
400 nm 10% Solids	PL6104-6141	PL6104-6142	

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