Protocol

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Coupling Protocol for Plain Latex Particles

Introduction

Agilent has been manufacturing polystyrene latex particles for more than 25 years, including plain latex microspheres and other variants. Agglutination type assays will require a biomolecule to be adsorbed to the surface of the beads. This protocol should be followed for successful coupling of biomolecules to plain latex particles. Agilent strongly recommends further optimization to achieve the best performance in your application.

Protein adsorption protocol

Materials required to perform the passive adsorption coupling protocol

- Reverse osmosis (RO) water
- Protein solution: 0.5 mL of protein prepared in RO water

Note: Titration of the protein amount is highly recommended. See step 3 and Table 2 for suggested starting protein concentrations and protein amounts.

- Blocking buffer: 0.1 M sodium borate (boric acid + NaOH), 0.5% Tween-20, pH 8.7
- Vortex mixer
- 2 mL Clear tubes
- Centrifuge
- Bottle roller

Before starting

- Consider the choice of latex beads, as they can be critical to performance depending on the application.
- Allow the reagents to reach room temperature.
- 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 a concentration measurement of the protein in RO water should be carried out in advance.

Procedure

1. Aliquot 10 mg (0.1 mL at 10.0% solids) of latex beads in a 2 mL clear tube.
2. Add 1 mL of RO water to the beads and mix the latex with the RO water 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 the 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. If this process is applied, solids content should be measured. Performing this step the day before the protocol is advised.

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

Bead Size (nm)	rpm at Room Temperature	Time (min)
100	15,000	120
200	15,000	50
400	15,000	30

3. Aspirate the RO water from the latex and add 500 µL of protein solution to the beads (see Table 2 for the recommended protein amount, depending on the latex size). Roll the beads for 60 minutes at room temperature.

Table 2. Recommended protein amounts for different latex bead 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: During the coupling process, it has been observed that the PBS and MES buffers promote bead agglomeration and subsequent precipitation of latex beads into the media. To prevent the loss of colloidal stability, avoiding MES and PBS-containing buffers is strongly recommended. Titration of the protein amount should be carried out to achieve optimal assay performance.

4. Centrifuge the tubes (see Table 1 for separation times, depending on the latex size), aspirate the supernatant, and add 1 mL of blocking buffer to the beads.
5. Roll the beads at room temperature overnight.
6. Centrifuge the tubes to separate and aspirate the supernatant.
7. Wash the beads with 1 mL of RO water, as indicated in step 2. Repeat this step twice.
8. Resuspend the beads in 1 mL of RO water or the desired storage buffer. For long-term storage, it is recommended to add 0.1% w/v of sodium azide as a preservative to avoid microbial growth.

Ordering information

PL-Latex Plain White Beads	15 mL	100 mL	1 L
50 nm 10% solids	PL6000-7101	PL6000-7102	PL6000-7103
100 nm 10% solids	PL6001-4101	PL6001-4101	PL6001-4103
200 nm 10% solids	PL6002-2101	PL6002-2102	PL6002-2103
300 nm 10% solids	PL6003-2101	PL6003-2102	PL6003-2103
400 nm 10% solids	PL6004-4101	PL6004-4102	PL6004-4103
600 nm 10% solids	PL6006-4101	PL6006-4102	PL6006-4103
800 nm 10% solids	PL6008-4101	PL6008-4102	PL6008-4103
1,000 nm 10% solids	PL6010-4101	PL6010-4102	PL6010-4103

PL-Latex Plain HiDye Beads	15 mL	100 mL	1 L
Blue			
300 nm 10% solids		Available on request	
400 nm 10% solids		Available on request	
800 nm 10% solids		Available on request	
1,000 nm 10% solids		Available on request	
Red			
200 nm 10% solids		Available on request	
300 nm 10% solids		Available on request	
400 nm 110% solids		Available on request	
800 nm 10% solids		Available on request	
Yellow			
600 nm 10% solids	PL6006-4161	PL6006-4162	PL6006-4163

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