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Latex beads behavior assay

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1 Works for me dx.doi.org/10.17504/protocols.io.8hnht5e

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ABSTRACT

Protocol for testing latex beads agreggation after resuspension in different buffers. It allows determining how beads storage conditions can influence their ability to move freely trhough a membrane.

GUIDELINES

In the following assay alittle ammount of beads suspension wil be eluted trhough a membrane. Dont pipette close one sample to each other, since it will lead to overlap of the developed elution fronts.

Beads separation by centrifugation can lead to difficult resuspension of them. Be sure that in each step beads are properly disperssed. Avoid excessive centrifugation time.

MATERIALS

NAME	CATALOG #	VENDOR
nitrocellulose membrane sheets size 210 m × 297 mm thickness 200 µm	Whatman® FF170HP Din A	Sigma Aldrich
Latex beads carboxylate-modified polystyrene fluorescent red 500nm average size.	L3280-1ML	

MATERIALS TEXT

- PBS-T Buffer pH = 7.4 (Tween-20 0.05%)
- Carbonate - Bicarbonate Buffer 100 mM pH = 9.6
- MES Buffer 50 mM pH = 5.9

- 2-20 µL micropipette
-100-1000 µL micropipette
- 1000 µL micropipette tips
- 200 µL micropipette yellow tips
- Eppendorf tubes for microfugue (1.5-2 mL)
- Microfugue for eppendorf tubes
- Permanent Marker

BEFORE STARTING

Prepare a piece of nitrocellulose paper big enough to hold the samples (4x4 cm will be enough).

Latex Beads Resuspension

1 Centrifugate 4 different aliquots of 40 µL 2.5 %wt. latex bead stock suspension at 15.000 rpm for 3 minutes. After centrifugation remove the supernatat slowly.

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2 Resuspend the beads in 100 µL of the four different buffers: distilled water, PBS-T, Bicarbonate Buffer and MES buffer.
For resuspending beads repeated pipetting it's highly recommended, aspiring and blowing out in the eppendorf tube.
Label the different eppendorfs with the buffer added.

Latex Beads Migration

3 For beads migration test, wait 10 minutes and pipette 10µL of each bead suspension on a piece of nitrocellulose membrane.
Separate each drop one to each other at least 8 mm.

4 Wait until the deposited sample has migrated completely, and circle on the plastic backing of the nitrocellulose membrane the outline of the sample eluted front.
Vissible results of beads retention in the membrane should be vissible at this point.

Latex beads reestablishment

5 Repeat step 1 and 2, resuspending all the beads in distilled water.

6 Repeat step 3-4 with the resuspended bead stocks.

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