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SPRI beads preparation

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Daniel Kitsberg[1,2], Miriam Adam[1,2], Naomi Habib[1,2]

[1]Edmond and Lily Safra Center for Brain Sciences, Hebrew University of Jerusalem;
[2]Rachel and Selim Benin School of Computer Science, Hebrew University of Jerusalem

Alon Chappleboim
Hebrew University of Jerusalem

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We use this protocol and it's working

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Abstract

SPRI is a technique for extracting nucleic acids from liquid mixtures.
This is a quick and cheap alternative to commerical SPRI beads for RNA extraction using the Sera-mag beads by GE, based on a **public protocol found here**.

The cost of this SPRI buffer is ~6$/10ml

Materials

MATERIALS

- NaCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**53014
- Trisodium citrate dihydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S1804
- nuclease free water
- Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles (Hydrophobic), 15 mL **GE Healthcare Catalog #**65152105050250
- Tween 20 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P1379
- HCL
- PEG 8000 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**81268

Before start

Make sure you have stock solutions:

- Nuclease-free water
- 5M NaCl
- 1N HCl
- 1M Trisodium citrate
- 50% PEG
- 10% Tween 20

SPRI buffer preparation

20m

1 **Prepare 50 ml RNA Wash Buffer:**
1 mM Trisodium citrate, 0.05% Tween 20, pH 6.4 @ 25 °C

5m

Add 30 mL nuclease free water

Add 50 µL 1M Trisodium citrate

Add 250 µL 10% Tween 20

Add 21 µL 1N HCl

Complete volume to 50 mL with nuclease free water

2 **Prepare 25 ml 50% PEG 8000 solution**

Weigh 12.5 g of PEG 8000 in a sterile 50 ml tube

Add no more than 14 mL of DDW

Rotate for about an hour until all the PEG is dissolved and the solution is homogeneous
Make up the volume to 25ml and allow the tube to rotate for another 10 minutes or so until a homogeneous solution is attained

Note

The 50% PEG solution is very viscous and takes a while to prepare

3 **Bead Preparation**

5m

1. Vortex the Sera-Mag beads thoroughly
2. Transfer 1 mL to a 1.5 ml microcentrifuge tube
3. Magnetize and discard the supernatant
4. Add 1 mL of RNA wash buffer
5. Remove the tube from the magnet and resuspend the beads by vortexing for at least 00:00:15
6. Spin down with a microcentrifuge, magnetize and discard the supernatant

Repeat steps 4 to 6 twice, for a total of 3 washes leaving the supernatant in the tube after the last wash

4 **Prepare** 50 mL **SPRI Buffer:**

10m

1mM Trisodium citrate, 2.5 M NaCl, 20% PEG 8000, 0.05% Tween 20, pH 6.4

25 °C

1. In a 50 mL conical tube, mix the ***base buffer***:
   - 4.672 mL nuclease-free water
   - 25 mL 5M NaCl
   - 28 µL 1N HCl
   - 50 µL 1M Trisodium citrate
1. Remove the supernatant from the beads, add 1 mL of ***base buffer*** to the bead tube and resuspend by vortexing for 00:00:15. Briefly spin down the liquid without pelleting the beads.
2. Add the washed beads to ***base buffer*** in 50ml tube. Cap and vortex for 00:00:30.
3. Add 20 mL of 50% PEG stock. Dispense slowly and allow the viscous liquid to slide down the inside walls of the pipette to ensure an accurate volume is added.
4. Add 250 µL 10% Tween 20
5. Cap the tube and mix by inversion

Note

The SPRI Buffer is ready to be used, and can be stored at 4 °C for at least two weeks (probably months). Verify pH before use.