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SPRI beads preparation

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1 Works for me dx.doi.org/10.17504/protocols.io.bes2jege

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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

NAME	CATALOG #	VENDOR
NaCl	53014	Sigma Aldrich
Trisodium citrate dihydrate	S1804	Sigma-aldrich
nuclease free water		
Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles (Hydrophobic), 15 mL	65152105050250	Ge Healthcare
Tween 20	P1379	Sigma
HCL		
PEG 8000	81268	Sigma Aldrich

BEFORE STARTING

Make sure you have stock solutions:

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

Citation: Daniel Kitsberg, Miriam Adam, Naomi Habib (04/13/2020). SPRI beads preparation. https://dx.doi.org/10.17504/protocols.io.bes2jege

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SPRI buffer preparation 20m

1 5m

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

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

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

3 5m

Bead Preparation

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

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10m

4 **Prepare 50 ml SPRI Buffer:**

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

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.

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