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SPRI bead mix

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1 Works for me dx.doi.org/10.17504/protocols.io.bnz4mf8w

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ABSTRACT

This protocol describes the preparation of stocks and buffers for inexpensive, convenient, and scalable DNA and RNA purification from aqueous solutions by solid-phase reversible immobilization (SPRI) on carboxylated paramagnetic beads. It also describes how to validate the effectiveness of the mixes before use.

The bead mixes described in this protocol are drop-in substitutes for AMPure XP and RNAClean XP beads (Beckman Coulter), but at about 1/100 of the cost (~$0.55/mL vs. $15–$70/mL at current Canadian prices).

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KEYWORDS

SPRI, AMPure, SPRIselect, RNAclean, Sera-Mag

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GUIDELINES

Chelating agents

EDTA and citrate may interfere with some enzymatic reactions by sequestering divalent cations such as Mg^{2+} and Mn^{2+}. These ions may damage nucleic acids or activate contaminating nucleases. On the other hand, sequestering these ions may interfere with downstream reactions that require them; if so, you can compensate by adding ions equimolar to the EDTA or citrate.

pH

The pH titrations for the buffers and bead mixes were calculated with the Python package ionize 0.8.0. They may be inaccurate for the bead mixes due to the very high ionic strengths of those solutions. Colour-change pH indicators will also be inaccurate for the same reason. A properly calibrated pH meter may be able to measure these solutions correctly. Keep in mind that the bead mix will be diluted during use when added to the sample to be purified, which will change the ionic strength and thus the pH.

Tween 20

Adding Tween 20 to the solutions described in the protocol is optional but provides multiple benefits. It reduces adhesion of nucleic acids to plastics, which is increased during SPRI due to the high ionic strength. This improves sample recovery. Tween 20 also reduces surface tension, which can pull beads off the pellet during supernatant removal. This effect becomes very useful if the pellet is very small. If Tween 20 is not compatible with your downstream processes or if foaming is a problem, replace its volume with nuclease-free water when mixing the solutions.

MATERIALS TEXT

Beads

- Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles (Hydrophobic), 15 mL **Ge Healthcare Catalog #65152105050250** Step 6.1

Chemicals (molecular biology grade)

Common:

- Sodium chloride (NaCl) **Contributed by users** In 2 steps
- Poly(ethylene glycol) avg. mol. wt. 8000 (PEG 8000) **Contributed by users** In 2 steps
- Polysorbate 20 (Tween 20) **Contributed by users** In 3 steps
- Hydrochloric acid (HCl), concentrated **Contributed by users** In 2 steps
- Water, nuclease-free **Contributed by users** In 3 steps

For DNA mix only:

- Tris(hydroxymethyl)aminomethane (Tris base) **Contributed by users** Step 6.10
- Disodium ethylenediaminetetraacetate dihydrate (EDTA) **Contributed by users** Step 6.10

For RNA mix only:

- Trisodium citrate dihydrate (sodium citrate) **Contributed by users** Step 6.10

Consumables

- 50 mL conical tubes
- 1.5 mL microcentrifuge tubes
- Disposable weighing vessels
- Disposable Pasteur pipettes
- Parafilm
- 0.22 µm syringe filters
- 10 mL disposable syringes
- 25 mL, 10 mL, 5 mL serological pipettes
- 1000 µL, 200 µL micropipette tips

Equipment

- Milligram-range balance
- Funnels
- Spatulas
- Heating plate
- Rotary mixer
- Microcentrifuge
- 25 mL graduated cylinder
- 50 mL volumetric flasks and stoppers
- 1000 µL, 200 µL adjustable-volume micropipettes
- Squirt bottle
- Magnetic separation block for 1.5 mL microcentrifuge tubes

Stock solutions prepared in this protocol

Common solutions:

- 1 N HCl
- 5 M NaCl
- 10% (v/v) Tween 20
- 50% (w/v) PEG 8000

DNA solutions:

- 1 M Tris base
- 0.1 M EDTA

RNA solution:

- 1 M trisodium citrate

SAFETY WARNINGS

The Sera-Mag bead suspension contains 0.05% sodium azide.

ABSTRACT

This protocol describes the preparation of stocks and buffers for inexpensive, convenient, and scalable DNA and

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RNA purification from aqueous solutions by solid-phase reversible immobilization (SPRI) on carboxylated paramagnetic beads. It also describes how to validate the effectiveness of the mixes before use.

The bead mixes described in this protocol are drop-in substitutes for AMPure XP and RNAClean XP beads (Beckman Coulter), but at about 1/100 of the cost (~$0.55/mL vs. $15–$70/mL at current Canadian prices).

Preparing stock solutions

1 Prepare at least **10 mL** **1 Molarity (M)** Hydrochloric acid (HCl), concentrated **Contributed by users** in a glass bottle from available concentrated stock.

2 In 50 mL volumetric flasks, prepare a separate 50 mL stock solution for each of the following components with the specified weights of solids.

Some gentle heating may be necessary. Ensure the solution comes back to room temperature before completing the volume to the mark on the flask. Store in 50 mL conical tubes.

Optional: filter the stocks with the syringes and filters to remove undissolved solids. It is strongly recommended to filter the solutions used for making RNA mix for sterilization.
Step 2 includes a Step case.
Common
DNA
RNA

step case

Common

5 M NaCl	14.610 g

3 Making 50 mL of 10% (v/v) Tween 20 stock

3.1 Place a labeled 50 mL conical tube on the balance and tare it.

3.2 With a new disposable Pasteur pipette, aspirate **1 mL** to **5 mL** of Polysorbate 20 (Tween 20) **Contributed by users** .

3.3 Slowly dispense the Polysorbate 20 (Tween 20) **Contributed by users** into the 50 mL conical tube to reach **5.475 g** .

3.4 Remove the tube from the balance and add **45.0 mL** of Water, nuclease-free **Contributed by users** with a 25 mL serological pipette.

3.5 Cap the tube and mix on a rotary mixer for **01:00:00** to dissolve the viscous liquid. 1h

4 Making 25 mL of 50% (w/v) PEG 8000 stock

This recipe can be scaled up with larger cylinders to make mixing easier, for example making 50 mL of solution in a 100 mL cylinder.

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4.1 Place the 25 mL graduated cylinder on the balance and tare it.

4.2 Weigh **12.5 g** of

Poly(ethylene glycol) avg. mol. wt. 8000 (PEG 8000) **Contributed by users** powder directly into the cylinder. It is recommended to use a fresh pair of gloves to reduce static charges that make the powder fly off the spatula.

4.3 Add no more than **14 mL** of Water, nuclease-free **Contributed by users**

Sodium chloride (NaCl) **Contributed by users** with a serological pipette on top of the PEG powder in the cylinder. The water level will reach over the 25 mL mark as the cylinder already contains about 20 mL of dry powder. Be sure not to fill the cylinder completely, as some air is required to make mixing possible. If the cylinder is too small, use a 50 mL one.

4.4 Seal the cylinder with a double layer of Parafilm.

4.5 Shake vigorously to suspend the powder in the water until there are no more lumps of dry solid sticking to the cylinder wall. It will be very viscous and clumpy.

4.6 Let the suspension stand at room temperature for at least **01:00:00** 1h to allow the solids to dissolve and the air bubbles to rise.

4.7 Remove the Parafilm and complete the volume with nuclease-free water to the **25 mL** mark.

4.8 Seal the cylinder again and mix well by inverting. The solution is very viscous and homogenizing it can take a while.

4.9 Transfer the solution to a 50 mL conical tube for storage. There will be some loss inside the cylinder but you need only **20 mL** for one batch of bead mix.

Buffer recipes 1m 30s

5 Nucleic acid elution and storage buffers

These solutions are used for preparing the beads before adding them to the mix. They are also useful for DNA and RNA elution and storage. It is possible to make concentrates of these solutions for convenience. Keep them refrigerated.
Step 5 includes a Step case.
DNA
RNA

step case

DNA

TE+Tween (10 mM Tris base, 1 mM EDTA, 0.05% Tween 20, pH 8.0 @ 25 °C)

Nuclease-free water	48.564 mL
Tris base, 1 M	0.500 mL

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Disodium EDTA, 0.1 M	0.500 mL
Tween 20, 10% (v/v)	0.250 mL
HCl, 1 M	0.186 mL

Ingredients for 50 mL

6 Nucleic acid binding bead mixes

6.1 Mix the
Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles (Hydrophobic), 15 mL **Ge Healthcare Catalog #65152105050250**
very well to resuspend.

6.2 Quickly transfer **1 mL** to a 1.5 mL microcentrifuge tube (the beads settle quickly).

6.3 Place the tube on a magnet stand until the supernatant is clear, about **00:00:30** . 30s

6.4 Remove and discard the supernatant.

6.5 Add **1 mL** of previously prepared "DNA buffer" or "RNA buffer", depending on the kind of bead binding mix you are preparing, to the bead pellet and close the tube.

6.6 Remove the tube from the magnet and resuspend the beads by vortexing for at least **00:00:15** . 15s
Spin down the liquid with a microcentrifuge.

6.7 Put the tube back on the magnet until the beads clear.

6.8 Remove and discard the supernatant.

6.9 **go to step #6.5 twice** , for a total of 3 washes with the appropriate buffer, leaving the supernatant in the tube after the last wash.

6.10 In a new 50 mL conical tube, combine the Water, nuclease-free **Contributed by users** , Sodium chloride (NaCl) **Contributed by users** and Hydrochloric acid (HCl), concentrated **Contributed by users** . For DNA, also add the Tris(hydroxymethyl)aminomethane (Tris base) **Contributed by users** and Disodium ethylenediaminetetraacetate dihydrate (EDTA) **Contributed by users** . For RNA, add only the Trisodium citrate dihydrate (sodium citrate) **Contributed by users** instead. Cap and mix well.

6.11 Remove the buffer supernatant from the bead tube still on the magnet.

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6.12 Add **1 mL** of incomplete binding buffer (prepared at step 6.10) to the bead tube on the magnet.

6.13 Remove the bead tube from the magnet and resuspend by vortexing for **00:00:15** (15s). Briefly spin down the liquid without pelleting the beads.

6.14 Add the washed beads to the incomplete binding buffer. Cap and vortex for **00:00:30** (30s).

6.15 With a 25 mL serological pipette, add **20 mL** of Poly(ethylene glycol) avg. mol. wt. 8000 (PEG 8000) **Contributed by users**. Dispense slowly and allow the viscous liquid to slide down the inside walls of the pipette to ensure an accurate volume is added.

6.16 Add the Polysorbate 20 (Tween 20) **Contributed by users**.

6.17 Cap the tube and mix by inversion gently but thoroughly, until the color appears homogeneous.

6.18 The bead binding mix is ready to be used or validated. Store at **4 °C**.

Step 6.18 includes a Step case.
DNA
RNA

Validation 1m 30s

step case

DNA

10 mM Tris base, 1 mM EDTA, 2.5 M NaCl, 20% PEG 8000, 0.05% Tween 20, pH 8.0 @ 25 °C

NaCl, 5 M	25.000 mL
Nuclease-free water	3.582 mL
Tris base, 1 M	0.500 mL
Disodium EDTA, 0.1 M	0.500 mL
HCl, 1 M	0.168 mL
PEG 8000, 50% (w/v)	20.000 mL
Tween 20, 10% (v/v)	0.250 mL
Sera-Mag bead suspension	1.000 mL

Ingredients for 50 mL

7 It is recommended to validate the bead mixes before use to ensure their effectiveness. They can be compared to AMPure XP or RNAClean XP, or to a previous batch of homemade mix. Validation can be done with DNA or RNA that is representative of a typical usage scenario, a DNA ladder (note that NEB ladders may contain modifications that make their SPRI behaviour unrepresentative of normal DNA), fragmented DNA across a range of sizes, or an RNA standard.
Step 7 includes a Step case.
DNA
RNA

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