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We use this protocol and it's working

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RNA cleanup with magnetic beads

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ABSTRACT

This protocol describes cleaning up RNA extracts with carboxylated magnetic beads and a PEG-NaCl buffer. It can also be used for volume reduction of a sample or buffer exchange after enzymatic reactions (e.g. DNAse treatment).

GUIDELINES

Follow general lab etiquette. Wear gloves to prevent contaminating the samples. Clean the workspace before starting with 80% EtOH.

MATERIALS TEXT

Materials required:
Below all materials needed for the protocol are listed. Vendors and part numbers are listed but interchangeable depending on the supply situation.

Chemicals:

Ethanol absolute
Ethanol absolute 99.8% **Fisher Scientific Catalog #11994041**
Hydrochloric acid fuming 37%
Hydrochloric acid fuming 37% **Sigma Aldrich Catalog #1003171011**
Tris ultrapure 99.9% Tris ultrapure 99.9% **Diagonal Catalog #A1086.1000**
EDTA disodium salt EDTA disodium salt Sigm
EDTA disodium salt **Sigma Aldrich Catalog #E5134-50G**
Tween 20 Tween 20 **Carl Roth Catalog #9127.1**
Sera-Mag SpeedsBeads
Sera-Mag SpeedBeads carboxylate modified particles **Sigma Aldrich Catalog #GE45152105050350**
Tri-Sodium citrate tri-Sodium citrate **Sigma Aldrich Catalog #1110371000**
Citric acid Citric acid **Sigma Aldrich Catalog #251275-100G**
Sodium chloride

Sodium Chloride Fisher BioReagents™ **Fisher Scientific Catalog #BP358-1**

PEG 8000

Polyethylene Glycol 8000 (PEG) **Fisher Scientific Catalog #10407773**

Labware:

125 mL Nalgene Wide-Mouth Bottle

Thermo Scientific Nalgene Wide-Mouth LDPE Bottle with Closure **Fisher Scientific Catalog #10044180**

Large magnet Neodyme magnet **Magnethandel Catalog #3935**

96-well plate magnet MM-Seperator M96 **Carl Roth Catalog #2141.1**

Hard-Shell PCR Plate

Hard-Shell 96-well PCR plate **BioRad Sciences Catalog #HSP9601**

1.2 mL Square Deep Well Storage Microplate

96 Square Deep Well Storage Plate U shaped bases **Azenta Life Sciences Catalog #4ti-0126**

Stock solutions:

1 L Tris stock solution | [M] 1 Molarity (m) | pH 8.5

- Add 121.14 g Tris ultrapure 99.9% to a beaker
- Adjust volume to 800 mL with ddH_2O
- Adjust pH to pH 8.5 with HCl
- Adjust volume to 1 L with ddH_2O
- Sterilize by filtering and store at Room temperature

1 L Tris stock solution | [M] 1 Molarity (m) | pH 8

- Add 121.14 g Tris ultrapure 99.9% to a beaker
- Adjust volume to 800 mL with ddH_2O
- Adjust pH to pH 8 with HCl
- Adjust volume to 1 L with ddH_2O
- Sterilize by filtering and store at Room temperature

1 L Tris stock solution | [M] 1 Molarity (m) | pH 7.5

- Add 121.14 g Tris stock solution to a beaker

- Adjust volume to 800 mL with ddH_2O
- Adjust pH to 7.5 with HCl
- Adjust volume to 1 L with ddH_2O
- Sterilize by filtering and store at Room temperature

500 mL trisodium citrate stock solution | 300 millimolar (mM) | 5

- Add 38.7 g tri-Sodium citrate to a beaker
- Adjust volume to 400 mL with ddH_2O
- Adjust pH to 5 with citric acid
- Sterilize by filtering and store at Room temperature

1 L wash buffer stock solution (50 millimolar (mM) Tris) 7.5

- Add 50 mL Tris stock solution 7.5 to a beaker
- Adjust volume to 1 L with ddH_2O
- Sterilize by filtering and store at Room temperature

1 L PEG-NaCl buffer (2.5 Molarity (m) NaCl , 20 Mass / % volume PEG 8000 , 1 millimolar (mM) tri-Sodium citrate 5 , 0.05 % (v/v) Tween 20) 5

- Add 200 g PEG 8000 to a sterile glass bottle
- Add 146.1 g NaCl
- Add 3.33 mL trisodium citrate stock solution 5
- Add 250 µL Tween 20
- Adjust volume to 1 L with ddH_2O
- Dissolve the PEG and NaCl by stirring and heating to 80 °C . The solution will become cloudy at this point.
- Let the solution cool down to Room temperature . A water bath may help speeding this up.
- Sterilize by filtering and store at 4 °C

Working solutions:

1 L TE minimum buffer (10 millimolar (mM) Tris ,

[M] 1 millimolar (mM) EDTA) pH 8

- Add 10 mL Tris stock solution pH 8 to a beaker
- Add 200 µL EDTA stock solution pH 8
- Adjust volume to 1 L with ddH_2O
- Sterilize by filtering and store at Room temperature

1 L wash buffer ([M] 10 millimolar (mM) Tris , [M] 80 % (v/v) Ethanol) pH 7.5

- Add 200 mL wash buffer stock solution to a beaker
- Adjust volume to 1 L with Ethanol absolute
- Sterilize by filtering and store at Room temperature

1 L elution buffer ([M] 10 millimolar (mM) Tris) pH 8.5

- Add 10 mL Tris stock solution pH 8.5 to a beaker
- Adjust volume to 1 L with ddH_2O
- Sterilize by filtering and store at Room temperature

100 mL RNA cleanup solution pH 5

- Add 2 mL Sera-Mag SpeedBeads barboxylate modified to a clean 125 mL Nalgene bottle
- Add 25 mL TE minimum buffer
- Shake the bottle to wash the beads
- Place the bottle on a large magnet for 00:05:00 to pellet the beads
- Discard the supernatant
- Add 25 mL TE minimum buffer
- Shake the bottle to wash the beads
- Place the bottle on a large magnet for 00:05:00 to pellet the beads
- Discard the supernatant
- Add 100 mL PEG-NaCl buffer
- Shake well to resuspend the beads
- Store at 4 °C

SAFETY WARNINGS

Reagents are potentially damaging to the environment. Dispose waste responsibly.

BEFORE START INSTRUCTIONS

Make sure all buffers are prepared before starting.
For more effortless pipetting let the bead solution adjust to Room temperature

Note

The protocol described here is designed for the use of 1.2 mL Square Deep Well Storage Microplates, but can also be done in tubes, PCR plates, strips, or any sufficient reaction vessel. The recommended shaking speeds are adjusted to the plates mentioned in the materials.

1 Shake the **RNA cleanup solution** until the beads are homogeneously resuspended

Note

The protocol described here is designed to clean up 100 µL of RNA sample. The ratio of sample to RNA cleanup solution used is 1:2. When cleaning up a different sample volume the amount of RNA cleanup solution should be adjusted to maintain the same ratio.

2 To 100 µL RNA sample add 200 µL RNA cleanup solution in a 1.2 mL Deep Well Storage Plate

3 To bind the RNA to the beads shake at 900 rpm, Room temperature , 00:05:00

> **Note**
>
> If the protocol is not done in a plate, mixing can also be accomplished by pipetting or vortexing.

4 Place the plate on a magnet to pellet the beads for 00:05:00 or until the mixture appears clear 5m

> **Note**
>
> Depending on the magnet and volume used separation times may vary and have to be adjusted accordingly.

5 Discard the supernatant by pipetting

6 With the plate still on the magnet, add 100 µL wash buffer to each sample

7 Incubate for at least 00:00:30 30s

8 Discard the supernatant by pipetting

9 go to step #6 and repeat once for a total of 2 washes

10 With the plate still on the magnet, incubate the plate for 00:05:00 at Room temperature to dry off residuals of wash buffer 5m

11 Add 100 µL of elution buffer to each sample

12 900 rpm, Room temperature , 00:05:00 to elute the RNA from the beads

13 Place the plate on a magnet to pellet the beads for 00:05:00 5m

14 Transfer 100 µL of the eluted RNA to a new storage plate. Store at -80 °C

Note

If bead-carryover is a concern, only 95 µL can be transferred for storage.

Note that not all sealing films are suitable for storage at -80 °C . If in doubt transfer the RNA to tubes for long-term storage.