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RNA cleanup with magnetic beads

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We use this protocol and it's working

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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

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% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #1003171011**

Tris ultrapure 99.9%  Tris ultrapure 99.9% **Diagonal Catalog #A1086.1000**

EDTA disodium salt EDTA disodium salt Sigma

 EDTA disodium salt **Merck MilliporeSigma (Sigma-Aldrich) Catalog #E5134-50G**

Tween 20  Tween 20 **Carl Roth Catalog #9127.1**

Sera-Mag SpeedsBeads

 Sera-Mag SpeedBeads carboxylate modified particles **Merck MilliporeSigma (Sigma-Aldrich) Catalog #GE45152105050350**

Tri-Sodium citrate  tri-Sodium citrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #1110371000**

Citric acid  Citric acid **Merck MilliporeSigma (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 **Bio-Rad Laboratories 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  1 Molarity (M)  8.5

- Add  121.14 g Tris ultrapure 99.9% to a beaker

- Adjust volume to 800 mL with ddH₂O
- Adjust pH to 8.5 with HCl
- Adjust volume to 1 L with ddH₂O
- 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 ddH₂O
- Adjust pH to 8 with HCl
- Adjust volume to 1 L with ddH₂O
- 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 ddH₂O
- Adjust pH to 7.5 with HCl
- Adjust volume to 1 L with ddH₂O
- Sterilize by filtering and store at Room temperature

500 mL trisodium citrate stock solution [M] 300 millimolar (mM) pH 5

- Add 38.7 g tri-Sodium citrate to a beaker
- Adjust volume to 400 mL with ddH₂O
- Adjust pH to 5 with citric acid
- Sterilize by filtering and store at Room temperature

1 L wash buffer stock solution ([M] 50 millimolar (mM) Tris) pH 7.5

- Add 50 mL Tris stock solution pH 7.5 to a beaker
- Adjust volume to 1 L with ddH₂O
- Sterilize by filtering and store at Room temperature

1 L PEG-NaCl buffer ([M] 2.5 Molarity (M) NaCl , [M] 20 Mass / % volume PEG 8000 ,
[M] 1 millimolar (mM) tri-Sodium citrate pH 5 , [M] 0.05 % (v/v) Tween 20) pH 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 μ L Tween 20
- Adjust volume to  1 L with ddH₂O
- 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 ,  1 millimolar (mM) EDTA)  8

- Add  10 mL Tris stock solution  8 to a beaker
- Add  200 μ L EDTA stock solution  8
- Adjust volume to  1 L with ddH₂O
- Sterilize by filtering and store at  Room temperature

 1 L wash buffer ( 10 millimolar (mM) Tris ,  80 % (v/v) Ethanol)  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 ( 10 millimolar (mM) Tris)  8.5

- Add  10 mL Tris stock solution  8.5 to a beaker
- Adjust volume to  1 L with ddH₂O
- Sterilize by filtering and store at  Room temperature

 100 mL RNA cleanup solution  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

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 μ L 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 μ L RNA sample add  200 μ L 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 μ L 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 μ L 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 μ L of the eluted RNA to a new storage plate. Store at -80 °C

Note

If bead-carryover is a concern, only 95 μ L 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.