EchoCLEAN™ RNA Kit – Protocol

for single-step removal of inhibitors and impurities from RNA solutions.

This protocol has been developed to eliminate impurities like inhibitors, salts and nucleotides from RNA solutions.

Materials and equipment needed.

- 80 to 110 µL of RNA sample for RNA samples less than 80 µL, dilute with RNAse-free water or Tris buffer to a minimum of 80 µL.
- Microcentrifuge with rotor for 1.5 mL and 2 mL reaction tubes.
  Important: Switch centrifuge to relative centrifugal force, rcf (x *g*); if this is not possible, please use formula below* to calculate the conversion of round per minute (rpm) into rcf.
- Vortexer
- One reaction tube (2 mL) per sample for column preparation
- One reaction tube (1.5 mL) per sample for elution and collection of the purified RNA.
- Pipets for 10 µL and 200 µL scale, corresponding pipet tips
- For fastest procedure (PROTOCOL 1): **Cap Puncher** (BioEcho product no. 050-001-001)

Preparation before starting.

- Set the microcentrifuge to **1,000 x g.**
- Important: **Switch to relative centrifugal force, rcf (x g, not rpm)**

Product use limitation

The EchoCLEAN RNA Kits are for research use only. They have not been registered or authorized to be used for diagnosis, prevention, or treatment of a disease.

* Most centrifuges offer the choice between rpm and *g*-force (rcf); if not, calculate the rpm matching the *g*-force using the formula: rpm = 1,000 x $\sqrt{(\frac{g}{1.12 \times r})}$, where r = radius of rotor in mm. and *g* the required *g*-force. E. g., with a radius of 150 mm, the corresponding rpm to 1,000 x *g* is approx. 2,400 rpm.

PROTOCOL 1: Purification using the Cap Puncher

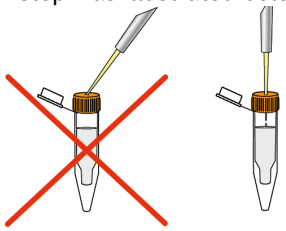
BioEcho Cap Puncher

Column preparation

1. Vortex the **EchoCLEAN RNA Spin Column** briefly and place into a **2 mL** reaction tube. Let stand for about 5 min.
2. Use of the **Cap Puncher** (scan QR code to watch a video): Punch a hole into the column cap and lift the column together with the **Cap Puncher** out of the 2 mL collection tube. Snap off bottom closure of the column and detach the Cap Puncher by twisting while pulling out.
   Place the punched spin column back into the 2 mL reaction tube.
3. Centrifuge for **1 min at 1,000 x g.** Discard the flow-through volume (void volume) collected in the 2 mL reaction tube.
4. Place the prepared spin column into a new **1.5 mL** reaction tube for elution and place back into the rack.
   Continue with cleanup of RNA.

Cleanup of RNA.

5. Transfer **80 – 110 µL of the RNA sample** to the prepared **EchoCLEAN Spin Column** from step 4 as illustrated below:



Insert pipet tip vertically through the hole in the column cap and pipet the sample slowly (~5 sec) into the column.

Note:

- Do not punch the resin bad with the pipette tip during loading of lysate!

6. Centrifuge **1 min at 1,000 x g.** The purified RNA elutes into the 1.5 mL elution tube and can be immediately applied in downstream applications.

The eluted RNA can be used immediately or stored at 4 °C or for long-term storage at – 80 °C. For spectrophotometric analysis, use **Low-TE Buffer** (T) as blank.

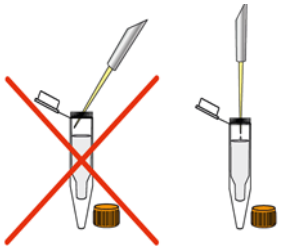
PROTOCOL 2: Purification **without** the use of a Cap Puncher

Column preparation

1. Vortex the **EchoCLEAN RNA Spin Column** briefly and place into a **2 mL** reaction tube. Let stand for 5 min.
2. Loosen the screw cap of the spin column **half a turn** and **snap off the bottom closure.**
   Important: Do not close the screw cap of the spin column. The screw cap must stay loosened **half a turn** to avoid vacuum generation. Place the column back into the 2 mL collection tube and both into the centrifuge.
3. Centrifuge for **1 min at 1,000 x g.** Discard the flow-through volume (void volume) collected in the 2 mL reaction tube.
4. Place the prepared spin column into a new **1.5 mL** reaction tube for elution of the sample RNA and place back into the rack.
   Continue with cleanup of RNA.

Cleanup of RNA.

5. Transfer **80 – 110 µL of the RNA sample** to the prepared **EchoCLEAN Spin Column** from step 4 as illustrated below.



Open cap and pipet the sample slowly (~5 sec) onto the center of the resin bed of the prepared spin column. Close screw cap and loosen again half a turn.
Important: Do not close the screw cap of the spin column tightly!

Note:

- Do not punch the resin bad with the pipette tip during loading of lysate!

6. Centrifuge **1 min at 1,000 x g**. The purified RNA elutes into the 1.5 mL elution tube and can be immediately applied in downstream applications.

The eluted RNA can be used immediately or stored at – 80 °C. For spectrophotometric analysis, use **Low-TE Buffer** (T) supplied with the kit as blank.

EchoCLEAN™ RNA Kit

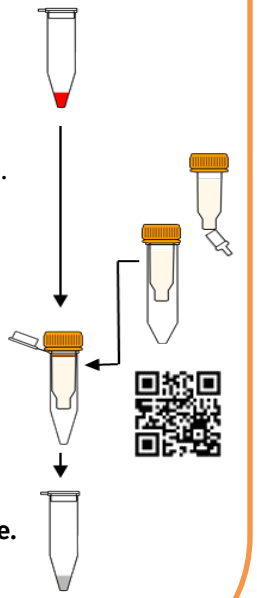
for single-step removal of AND impurities from RNA solutions

Product no.	020-002-050-010 (10)	020-002-050-050 (50)
Kit contents	EchoCLEAN RNA Spin Columns and Low-TE Buffer	

Quick PROTOCOL (please read protocol first)

Column preparation.

- Vortex **EchoCLEAN spin column** and place in a **2 mL** tube. Let stand for 10 min.
- Punch a hole in the cap with the Cap Puncher, and break off bottom closure (scan QR code to watch a video).
- Place spin column back into 2 mL tube.
- Centrifuge **1 min at 1,000 x g** to elute column buffer.
- Place column in a **1.5 mL** tube.



Cleanup of RNA.

- Transfer **RNA sample (max. 110 µL)** by pipetting **slowly** through cap hole (scan QR code to watch a video)
- Centrifuge **1 min at 1,000 x g** to elute RNA into **elution tube.**
- Eluted RNA is ready to use.

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