We drive RNA research with tailored molecular tools

PROTOCOL

PRODUCT DESCRIPTION

The riboPOOL™ cleanUP module is an RNA clean-up kit that efficiently purifies your rRNA depleted RNA sample prior to downstream applications.

The riboPOOL™ cleanUP module is provided together with the riboPOOL™ kit.
For riboPOOL kits, please refer to our riboPOOL Kit manual provided.

Why use the riboPOOL cleanUP module?
RNA samples that have been subject to rRNA depletion **must** be purified before sequencing library preparation to remove salts and buffer concentrates.

How to does the riboPOOL cleanUP module work?
The riboPOOL cleanUP module uses solid-phase paramagnetic clean-up beads that hybridize to RNA. The final product is then eluted from the beads for further processing.

MATERIALS PROVIDED

- Clean-up beads

Additional materials and equipment required for RNA purification (not provided)

- Magnetic tube rack
- 96-well-plate for 96 reaction kits (if preferred instead of tubes)
- Fresh 70% ethanol, RNase free
- Nuclease-free water

PURIFICATION PROTOCOL FOR 90 µL RNA SAMPLE

The workflow with the riboPOOL™ cleanUP module can be completed in ~30 min, is enzyme-free, and compatible with high-throughput automation.

1. Add 90 µl of your RNA sample (e.g. rRNA depleted RNA) in an appropriate tube.

2. Add 162 µl **Clean-up Beads (CB)** to each sample. Be sure to resuspend the **beads** completely before adding them by flicking the tube or pipetting.

   Note: The volume of of Clean-up Beads for a given reaction can be calculated from the following equation: Volume of Clean-up Beads per reaction = 1.8 x reaction volume.

3. Mix solution with 6-8 pipetting strokes and let the tube incubate at room temperature for 5 minutes. This step binds RNA products to the magnetic beads. Vortex is not recommended.

4. Separate the **Clean-up Beads** by placing the tube on a magnet for 5 minutes. The separation time is dependent on size. Larger reactions will take longer to separate. Wait for the solution to clear before proceeding to the next step.

5. With the tube still on the magnet, slowly remove and discard the supernatant without disturbing the pellet.

6. With the tube still on the magnet, add 500 - 1000 µl of 70% ethanol (70% ethanol and 30% H_2O) to each

sample and incubate for 30 seconds at room temperature. Carefully remove and discard the ethanol without disturbing the pellet.

Repeat for a total of three washes. Note: Be sure to remove all ethanol from the tube as it may contain residual contaminants. The wash solution should completely cover the pellet in the tube.

7. Let the reaction tube air-dry for 10 minutes on the magnet. The tube should air-dry until the last visible traces of ethanol evaporate. Be careful and do not over dry the pellet as it may lead to lower recovery.

8. Remove the tube from the magnet. Elute the purified RNA from the beads by adding at least 30 µL of nuclease-free water. Resuspend the beads by pipetting up and down several times. Elution volume can be modified if higher/lower RNA concentration is needed, but it's not recommended to eluate in less than 10 µl.

9. Incubate for 2 min. at room temperature.

10. Place the tube back on the magnet and wait for 5 minutes or until the **Clean-up Beads** clear from solution.

11. Transfer the eluate containing your purified RNA sample to a clean tube.

HANDLING TIPS

- Be sure to resuspend clean-up beads completely before using them. The beads should appear homogenous and consistent in color.
- Avoid touching the pellet containing your RNA when removing the supernatant during the wash step.
- Make sure all the supernatant is removed after the last wash step.

AVAILABLE FORMATS AND CONTENTS OF THE RIBOPOOL CLEANUP MODULE

Kit size	Bottle provided for (CB)	Clean-up Beads
6 reactions	1x 2mL tube	1.05 ml
12 reactions	1x 8mL tube	2 ml
24 reactions	1x 8mL tube	4 ml
96 reactions	1x 30mL tube	16 ml

STORAGE TEMPERATURE

The riboPOOL cleanUP module can be stored at 4-8°C **(do not freeze)** at least for 1 year.