

## 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.

*This protocol is for the use of riboPOOL™ cleanUP module alone.*

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 does the riboPOOL cleanUP module work?**

The riboPOOL cleanUP module uses Nucleic Acid Binding M-PVA magnetic beads, that hybridize to RNA. The final product is eluted from the beads for further processing.

## Materials provided:

- Clean-up Beads
- Binding Buffer
- Wash Buffer
- Nuclease-free Water

## 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)

## Purification Protocol for 100 µl RNA Sample:

The workflow with the riboPOOL™ cleanUP module can be completed in ~30-45 min, is enzyme-free, and compatible with high-throughput automation.

1. Add up to 100 µl of your RNA sample (e.g. rRNA depleted RNA) in an appropriate tube.
2. Add 10 µl **Cleanup Beads (CB)** to each sample. Be sure to resuspend the **Beads** completely before adding them.
3. Add 200 µl **Binding Buffer (BB)** to each sample.
4. Mix with 4 - 5 pipetting strokes and incubate for 5 min. at room temperature.
5. Following incubation, separate the **Cleanup Beads** by placing the tubes on a magnet. Once the beads have been attracted to the magnet, discard the supernatant.
6. Remove the tube from the magnet and add 200 µl **Wash Buffer (WB)** to each sample.
7. Thoroughly resuspend the beads in the **Wash Buffer** by pipetting the bead pellet up and down.
8. Separate the **Cleanup Beads** by placing the tube on a magnet. Discard the supernatant and then remove the tube from the magnet.
9. Wash the beads once more with 200 µl **Wash Buffer** (repeat steps 6-8).

*It is important to completely remove all the supernatant after the last wash step.*

10. Remove the tube from the magnet and allow the particles to air-dry for approx. 10 minutes at room temperature until **Cleanup Beads** appear dry.
11. Add 20 -50 µl of nuclease-free water to the beads and resuspend thoroughly by pipetting
12. Elute for 5 min. at room temperature with occasional agitation.
13. Following elution, separate the **Cleanup Beads** by placing the tube on a magnet and wait for 2 min. until all the beads have been attracted to the magnet.
14. Transfer the eluate containing your purified RNA sample to a clean tube.

## Handling tips:

- Clean-up beads do not have to be cooled; the workflow can be completed at room temperature.
- Be sure to resuspend clean-up beads completely before using them.
- 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.

## Benefits of using the riboPOOL cleanUP module:

- **Single-product solution**  
With the addition of the riboPOOL cleanUP module, you have everything you need to prepare your RNA samples. There is no need to buy extra reagents or beads for the RNA clean-up step anymore.
- **Faster and more convenient**  
Our easy-to-follow protocol has been optimized for faster results and can be completed within ~30-45 minutes.
- **Fully automatable and scalable**  
The clean-up beads' high magnetite content allows for fast magnetic separation, even in large sample volumes making it easy to automate the workflow.
- **High binding capacity**  
µm sized beads present a high active surface per volume and allow efficient adaptation to specific applications.
- **Reproducible results**  
Polydisperse particles for a uniform reproducibility of magnetic separation.
- **Specific RNA binding**  
Hydrophilicity of the PVA surface, leading to low non-specific protein binding.

## Available formats and contents of the riboPOOL cleanUP module:

Kits	Water	Clean-up Beads	Binding Buffer	Wash Buffer
6 reactions	1x 2mL tube	140 µl	1x 8mL tube	1x 8mL tube
12 reactions	1x 2mL tube	140 µl	1x 8mL tube	1x 8mL tube
24 reactions	1x 2mL tube	280 µl	1x 8mL tube	1x 60ml tube
96 reactions	1x 2mL tube	1100 µl	1x 30mL tube	1x 60ml tube

## Storage temperature:

All reagents included in the riboPOOL cleanUP module can be stored at room temperature at least for 1 year.