Auto-Mag® RNA-Pure

Cat # AMD-S001 Version 2.0

Protocol for Separation and Recovery of Large RNAs and Small RNAs

The following protocol exemplifies for enriched RNA below 200nt into the "small RNA" fraction while RNA above 200nt is enriched in the "large RNA" fraction from the 50ul Total RNA samples.

Materials and Equipment to be supplied by User:

- 100% ethanol
- Nuclease-free Elution Buffer, (diH_2O, 10mM Tris-HCl pH 8.0 or TE buffer)
- For 96 well format: 96 well cycling plate
- Well calibrated pipettor and Disposable pipette tips
- ~1.5 ml RNase free tubes
- Appropriate magnetic separation device compatible with 96-well PCR plate or tubes.

Before Starting

- Prepare fresh 80% ethanol (Prepare from absolute ethanol. Do not use denatured alcohol).
- Bring the Auto-Mag® RNA-Pure to room temperature for at least 30 min before use.
- Suspension Auto-Mag® RNA-Pure reagents by vortexing.

Procedure

1. Completely float the Auto-Mag® RNA-Pure reagent.

2. For using the 96-well plate, a starting sample volume of 50µl is recommended. If sample volume is less than 50 ul, bring sample volume up to 50µl with nuclease-free water or the Elution Buffer.

3. Label the plate as "Large RNAs". Add 50µl Auto-Mag® RNA-Pure reagent into the wells containing 50µl total RNA sample. Mix thoroughly by pipetting up and down 10 times.

4. Incubate the mixture for 10 minutes at room temperature to allow the large RNAs are bound to Auto-Mag® RNA-Pure beads.

5. Place the Large RNAs sample plate on the magnetic separation device and allow the magnetic beads to settle on the magnet for 5-10 minutes or until the supernatant turns completely clear.

6. With the Large RNAs sample plate still on the magnet, transfer ~100µl clean supernatant (including the small RNAs) to the new plate (label this new plate as "Small RNAs).

Note: Do not disturb the attracted beads while transfer the supernatant.

7. To recover large RNAs, go to step 13: RNA washing steps

Note: Discard the large RNA plate if there is no need for large RNAs.

8. To recover small RNAs, use the new plate labeled "small RNAs" and continue to step 9.

9. Completely float the Auto-Mag® RNA-Pure reagent again.

10. Add 40µl Auto-Mag® RNA-Pure reagent and 50µl 100% isopropanol into the well of small RNAs plate containing the clean supernatant from step 6. Mix thoroughly by pipetting up and down 10 times.

11. Incubate the mixture for 10 minutes at room temperature to allow the small RNAs are bound to Auto-Mag® RNA-Pure beads.

12. Place the small RNAs sample plate on the magnetic separation device and allow the magnetic beads to settle on the magnet for 5-10 minutes or until the supernatant turns completely clear. Carefully remove and discard the supernatant by pipetting.

Note: Do not disturb the attracted beads while remove and discard the supernatant.

13. RNA wash steps

14. Keep the sample plate on the magnet and add 300µl of 80% ethanol to each well and incubate for 10 seconds at room temperature. Carefully remove and discard the supernatant by pipetting.

Note: Do not disturb the attracted beads while aspirating the supernatant. Significant bead loss will result in reduced yield.

15. Repeat steps 14 for second 80% ethanol wash.

16. Keep the sample plate on the magnetic separation device and air dry the magnetic beads by incubating sample tube at room temperature for 5 minutes.

Note: It is critical to completely remove all traces of alcohol but take caution in not over drying the beads as this will reduce the yield.

17. Remove the sample plate from the magnetic separation device. Add 20-50µl of RNase/DNase free elution buffer, (reagent grade water, Tris-HCl pH 8.0 or TE buffer) to each sample and mix by pipetting up and down 10 times. Incubate for 5 minutes at room temperature.

Note: Pre-warming the elution buffer at 55°C can increase the yield.

18. Place the sample plates back on the magnetic separation device and wait 2 minute or until the magnetic beads clear from solution.

19. Transfer the eluate (the Large RNAs (> 200nt) from large RNAs plate; or Small RNAs (< 200nt), from small RNAs plate) to an appropriate storage vessel.

20. The ultra-pure RNAs are ready for subsequent applications, or store at -80°C for long term storage.