AMD Biotech Inc.
Advanced Molecular Detection

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# Auto-Mag® RNA-Pure

Cat # AMD-S001 Version 2.0

## Basic Protocol for Total RNA Clean-up

### Materials and Equipment to be supplied by User:

- 100% ethanol
- Nuclease-free Elution Buffer, (di$H_2O$, 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. Add the appropriate volume of Auto-Mag® RNA-Pure reagent into the reaction vessels containing 10-100µl RNA sample according to the instructions in the table below.

| Sample Volume (ul) | Auto-Mag® RNA-Pure Volume (ul)* |
|---|---|
| 10 | 18 |
| 20 | 36 |
| 25 | 45 |
| 50 | 90 |
| 100 | 180 |

** Formula used to calculate the volume of Auto-Mag® RNA-Pure reagent needed for RNA sample: Auto-Mag® RNA-Pure reagent volume per reaction = 1.8 X sample volume.*

3. Mix thoroughly the Auto-Mag® RNA-Pure reagent and the samples by pipetting up and down 10 times. Incubate the mixture for 10 minutes at room temperature.

4. Place the sample reaction vessels on the 96 well, or appropriate 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 aspirating the supernatant. Significant bead loss will result in reduced yield.*

5. With the sample reaction vessels still on the magnet and add 300µl of 80% ethanol to each well and incubate for 30 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.*

6. Repeat steps 5 for second 80% ethanol washes.

7. Keep the sample reaction vessels 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.*

8. Remove the sample reaction vessels 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.*

9. Place the sample reaction vessels back on the magnetic separation device and wait 2 minute or until the magnetic beads clear from solution.

10. Transfer the eluate (cleared supernatant) to an appropriate storage vessel and keep at -80°C for long term storage, or for subsequent applications.