AMD Biotech Inc.
Advanced Molecular Detection

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Auto-Mag® RNA-Pure

Cat # AMD-S001 Version 2.0

❖ Magnetic beads-based reagent for manual and automated RNA or cDNA Clean up

Highlights

- Efficient Purify RNA and cDNA from common enzymatic reactions using paramagnetic bead-based chemistry.
- RNA or cDNA Recover rate >90% and elute in as little as 10 µl.
- Efficiently removes unincorporated nucleotides, aborted transcripts, enzymes and buffer components
- RNA or cDNA is ready for all downstream applications including NGS, RNA-Seq library preparations, RT-qPCR, and hybridization, etc.
- No centrifugation or filtration needed. Manual and automation-friendly sample processing in 96 formats

Protocol Contents

- Product Highlights.....1
- Product introduction and information.....2
- Kit Contents, Storage, Stability.....2
- Basic Protocol for Total RNA Clean-up.....4
- Protocol for Separation and Recovery of Large RNAs and Small RNAs.....6
- Troubleshooting.....8

Disclaimers and Safety Information

This kit is designed for research purposes only. The all biological samples are considered potentially infectious. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs).

Trademarks

Product Introduction

Auto-Mag® RNA-Pure consists of RNase free AMD's paramagnetic particles and is optimized chemically for high-throughput RNA or cDNA cleanup and concentration after any enzymatic reaction: including RNA synthesis in vitro transcription, DNase I treatment, capping and labeling, and even after other purification methods such as phenol/chloroform extraction. The RNA after cleanup is beneficial for the following standard protocols such as RNA labeling, capping, Proteinase K treatment, DNase I treatment, RNA-Seq, and RT-qPCR. This protocol enables the recovery of micro RNA (miRNA), small RNA, and total RNA from the enzymatic reactions by concentrating the RNA from a diluted sample.

RNA can be cleaned up in various ways, including phenol/chloroform extraction, lithium chloride precipitation, agarose gel electrophoresis, or spin columns isolation. Magnetic bead-based cleanup offers a new more efficient tool to clean up the RNA and it can be used for both manual procedure as well as automatic liquid handling instruments to clean up and/or concentrate the sample of interest.

Auto-Mag® RNA-Pure also can be used for PCR/DNA clean-up and DNA size selection for NGS library preparation processing. Please reference protocols of AMD Auto-Mag® PCR-Pure.

Applications

- PCR and RT-PCR reactions
- Transfection for RNAi Experiments
- Probes for microarray or microarray
- cDNA synthesis and labeling
- In vitro RNA synthesis

Kit Contents

Product Number	AMD-S001-5	AMD-S001-50	AMD-S001-250	AMD-S001-500
Auto-Mag® RNA-Pure	5 ml	50	250 ml	500 ml

Storage and Stability

Auto-Mag® RNA Pure is shipped at room temperature and is guaranteed for at least 12 months from the date of purchase when the kit is stored at 2-8°C after received. It should never be frozen at any time.

Working in RNase Free Conditions

RNases are present everywhere and some general precautions should be followed in order to avoid the introduction of contaminating nucleases during the Auto-Mag® RNA-Pure procedure. The most common sources of RNase contamination are hands, dust particles, and contaminated laboratory instruments, solutions and glassware. The following procedures should be followed to limit RNase contamination when working with RNA:

- Always wear gloves while working and change gloves frequently
- Refrain from using reagents, consumables and equipment that are in common use for other general lab processes
- Use dedicated RNase free equipment such as pipettes, pipette tips, gels boxes, etc.
- Work in a separate room, fume hood or lab space if available
- Use plastic, disposable consumables that are certified RNase free
- Purchase reagents, such as commonly used buffers and water, that are certified RNase free. Prepare small individual aliquots of such buffers to avoid repeated transfer out of stock buffers. This lowers the risk of contamination of the stock solution
- Wipe down work surfaces with commercial RNase inhibiting surfactant solutions or 70% ethanol before starting work

Basic Protocol for Total RNA Clean-up

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. 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.

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.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via: Phone: 1-404-259-6276 (in US), Email: support@amdbiotech.com

Symptoms	Possible Causes	Comments
Low RNA yield	Reagents added incorrectly	Check protocol to ensure correct buffer reconstitution, order of addition of buffers and ethanol, and eluents.
	Insufficient mixing of reagents	Ensure each step is thoroughly mixed with RNA sample and Auto-Mag® RNA-Pure reagent
	Elution buffer volume insufficient	Bead pellet must be covered completely with elution buffer. Larger elution volumes, multiple elution's, and longer incubation times can increase yield of RNA, but will dilute the sample and may increase processing times
	Incomplete elution during prep	Do not dry beads longer than 15 min at room temperature. Over drying of beads may result in lower elution efficiencies.
Purified RNA is Degraded	RNase contamination	In order to avoid RNase contamination during RNA cleanup, make sure to work on a clean lab bench, wear gloves and use disposable RNase-free pipet tips and microfuge tubes. Keep all kit components tightly sealed when not in use.
	Improper storage of RNA	Purified RNA should be used immediately in downstream applications or stored at -70°C.
Low Performance of RNA in Downstream Steps	Salt and/or ethanol carry-over	Ethanol and salt remaining after the washes may inhibit downstream applications. Use care to ensure traces of salt and ethanol are not carried over in the eluted RNA.
	DNA contamination	DNA removal may be necessary for certain applications. Incubate RNA sample with DNase I and cleanup RNA.