AMD Biotech Inc.
Advanced Molecular Detection

Product Manual

Auto-Mag® RNA-Pure

Version 2.1

Magnetic beads-based reagent for manual or automated RNA or cDNA purification and concentration

Catalog Number: S008-01, S008-02, S008-03, S008-04

Contents

Disclaimers and Safety Information

This kit is designed for research use only. All biological samples are considered potentially infectious. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). MSDS can be downloaded from the "Product Documents" tab when viewing the product kit. Download MSDS at www.amdbiotech.com. Information in this document is subject to change without notice.

Product Introduction

Auto-Mag® RNA-Pure provide a fast and simple paramagnetic bead-based reagent for RNA cleanup and concentration after any enzymatic reaction (including in vitro transcription, DNase I treatment, capping and labeling) and after other purification methods such as phenol/chloroform extraction. The kit also can be used to selectively recover different sizes range RNA from total RNA samples in NGS based procedures by using supplementary protocol.

Auto-Mag® RNA-Pure can be used for manual procedures as well as for adapting to automated liquid handling instruments for high-throughput sample cleanup. The highly purified RNA can be used directly for downstream applications, such as PCR and RT-PCR reactions, RNase protection assays, Transfection for RNAi experiments, Antisense RNA (aRNA) amplification, Probes for microarray or microarray, cDNA synthesis and labeling, as well as RNA-Seq library preparations, etc.

Features:

- Wors with RNA & cDNA
- No centrifugation step, no filtration step
- Compatible with manual and automated processing
- Rapid and reliable clean-up total RNA, including miRNA, siRNA, and aRNA
- Complete removal of salts, unincorporated primers, and dNTPs
- Efficient purification of both large and small RNAs, and RNA size selection
- Used in RNA-Seq library preparations.

Kit Contents

Product Number	S008-01	S008-02	S008-03	S008-04
Auto-Mag® RNA-Pure	5 ml	50 ml	250 ml	500 ml

Storage and Stability

Auto-Mag® RNA Pure is shipped at room temperature and is stable for at least 18 months from the date of manufacture when stored at 2-8°C. Contents of the kit should never be frozen at any time.

Preparation of Reagents

1. Prepare 80% Ethanol for RNA Wash. (Prepare from absolute ethanol. Do not use denatured alcohol).

Ethanol is hygroscopic. When opened the ethanol will both evaporate and absorb water over time. Fresh prepare 80% ethanol then keep cover tight and use within one week.

2. RNase free Molecular biology grade water, Tris (10 mM, pH 8.0) or TE (10 mM Tris, pH 8.1 mM EDTA) for RNA elution

Additional Information

1. Specifications

Features	Specification
Isolation Technology	Magnetic Beads
Sample Sources	RNA, cDNA
Starting amount	Scalable
Recovery	>90% recovery
Downstream Application	RT-PCR, Probes for microarray, cDNA synthesis and labeling, RNA-Seq library preparations, etc.
Processing format	Automated or Manual
Storage	2°C - 8°C

2. Working in RNase Free Conditions

RNA purity and integrity are essential for downstream applications. RNA can be degraded by RNase A, which is a highly stable contaminant found in any laboratory environment. Some general precautions should be followed to avoid the introduction of contaminating nucleases especially during wash and elution steps. The most common sources of RNase contamination are hands, dust particles, contaminated laboratory equipment, 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 in the stock solution.
- Wipe down work surfaces with commercial RNase inhibiting surfactant solutions or 70% ethanol before starting work.
- Keep the RNA on ice after extraction and while working with it.
- Store the extracted RNA at -20°C. For long term stability, keep the RNA at -80°C.

Auto-Mag® RNA-Pure Protocols

Following protocols that applies to the desired purpose and format.

- Protocol for Total RNA Clean-up (single-tube or 96-well format)
- Protocol for Separation and Recovery of Large RNAs and Small RNAs (96-well format

Materials and Equipment to Be Supplied by User

- Single-tube format: Nuclease-free 1.5-2.0 ml tube, and Magnetic Rack Separator for tube. (Any vendor of choice).
- 96-well format: 96 well thermal cycling plate, or 500µl round bottom microtiter plate, or 1.2 ml deep well microtiter plate and appropriate magnetic separation device
- Laboratory mixer, vortex, or equivalent.
- 80% ethanol (Prepare from absolute ethanol. Do not use denatured alcohol).
- 100% Isopropanol
- RNase/DNase free Elution buffe (AMD-B232 www.amdbiotech.com), or molecular biology grade RNase/DNase free water or 10 mM Tris-HCl, pH8.5.
- Well calibrated pipettor and Disposable pipette tips.

Before Starting

- Prepare 80% Ethanol for DNA wash steps according to the instructions of Preparation Reagents on page 3.
- Bring the Auto-Mag® RNR-Pure bottle to room temperature for at least 30 minutes before use.
- Suspension Auto-Mag® RNA-Pure Select by vortex.

Protocol for Total RNA Cleanup (single-tube or 96-well format)

This Protocol will purify and recover all of RNA molecules including miRNA, siRNA, and aRNA from pre-purified total RNA or enzyme reactions.

1. Shake thoroughly the Auto-Mag® RNA-Pure reagent to fully resuspend the magnetic beads.

2. Confirm the volume of RNA sample and transfer the sample to a 1.5-2.0 ml tube, or the well of 96 well plate (96 well format)

Note: PCR plates generally have a maximum volume of 200µl. If the RNA sample volume multiplied by 5 exceeds 200µl, transfer sample to a 500ul round plate or a 1.2 ml deep- well plate.

3. Add the appropriate volume of Auto-Mag® RNA-Pure reagent and 100% isopropanol according to the sample volume shown in table.

Sample Volume (µl)	Auto-Mag® RNA-Pure Volume (µl)*	100% Isopropanol (µl)**
10	18	20
25	45	50
50	90	100
100	180	200
(Volume of Auto-Mag® RNA-Pure reagent needed per reaction) = 1.8 X (RNA sample volume)		***Volume of isopropanol needed per reaction = 2 X (RNA sample volume)*

4. Mix the total reaction volume by pipetting up and down 10 times or vortex for 30 seconds.

   Note: Pipette mixing is preferable. If a 96 well plate and vortex is used, the plate must be sealed with a plate seal before vortex.

5. Incubate mixed sample at room temperature for 5 minutes at least.

   Note: For >50µl sample, longer incubation time is recommended. For the purification of single stranded cDNA, an incubation of up to 20 minutes can increase recovery.

6. Place the sample reaction tubes or plate on a compatible magnetic separation device for 10 minutes or until the solution clears. Beads will pull to the side of the well.

7. With the sample plate or tube still on the magnet, remove and discard the supernatant by pipetting. Do not disturb the attracted beads.

8. With the samples still on the magnet, add 200µl of 80% ethanol to each sample and incubate at room temperature. for 30 seconds. Mixing is not necessary.

   Note: If the total volume of sample plus reagent exceeds 200µl, the volume of 80% ethanol for each wash should be at least 5 x of original RNA sample volume. for example: 100ul RNA sample need 100 x 5=500ul 80% ethanol for washing.

9. With the samples still on the magnet, remove and discard all the liquid. Do not disturb the attracted beads.

10. Repeat steps 8-9 for a second 80% ethanol washes.

11. Keep the sample on the magnet, and air dry the magnetic beads at room temperature for 5 minutes. Remove any residue liquid with a pipettor.

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

12. Remove the sample from the magnetic separation device. Add 20-50µl of molecular biology grade RNase/DNase free water or 10 mM Tris-HCl, pH8.5 to each sample.

    Note: The Elution volume should be large enough so that the liquid level is high enough on the magnet for the

beads to settle.

13. Mix the elution buffer by pipetting up and down 10 times to resuspend the beads and incubate at room temperature for 5 minutes

14. Place the sample back on the magnetic separation device and wait 5 minute or until the magnetic beads are completely cleared from solution.

15. 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 (96well format)

The following protocol applies to separate and recover "large RNA, > 200nt", and "Small RNA, <200nt" from the 50µl total RNA samples.

1. Shake thoroughly the Auto-Mag® RNA-Pure reagent to fully resuspend the magnetic beads.

2. Add 50µl RNA sample to the well of 96-well plate. If sample volume is less than 50µl, 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 the total reaction volume by pipetting up and down 10 times.

4. Incubate the sample plate at room temperature for 10 minutes to allow the large RNAs to bound to the beads.

5. Place the "Large RNAs" plate on the magnetic separation device for 10 minutes or until the solution clears. Beads will pull to the side of the well.

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

   Note: Do not disturb the attracted beads while transferring the supernatant. Significant bead carry will result in Larger RNA mixing with the small RNA portion.

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. Shake thoroughly the Auto-Mag® RNA-Pure reagent again to fully resuspend the magnetic beads.

9. 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 the total reaction volume by pipetting up and down 10 times.

10. Incubate the plate at room temperature for 10 minutes to allow the small RNAs to bound to the beads.

11. Place the "Small RNAs" plate on the magnetic separation device for 10 minutes or until the solution clears. Beads will pull to the side of the well.

12. With the “Small RNAs” plate still on the magnet, 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.

13. RNA washing steps: With the sample plate still on the magnet, add 200µl of 80% ethanol to each sample and Incubate at room temperature for 30 seconds. Mixing is not necessary.

14. With the sample plate still on the magnet, remove and discard all the liquid by pipetting. Do not disturb the attracted beads.

15. Repeat steps 13-14 for a second 80% ethanol wash.

16. Keep the sample plate on the magnet, and air dry the magnetic beads at room temperature for 5 minutes. Remove any residue liquid with a pipettor.

    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 molecular biology grade RNase/DNase free water or 10 mM Tris-HCl, pH8.5 to each sample.

    Note: The Elution volume should be large enough so that the liquid level is high enough on the magnet for the beads to settle.

18. Mix the elution buffer by pipetting up and down 10 times to resuspend the beads and incubate at room temperature for 5 minutes

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

19. Place the sample back on the magnetic separation device and wait 5 minute or until the magnetic beads are completely cleared from solution.

20. Transfer the eluate: the Large RNAs (> 200nt) from “large RNAs” plate; or Small RNAs (< 200nt), from “Small RNAs” plate to an appropriate storage vessel. The ultra-pure RNAs are ready for subsequent applications or stored at -80°C for long term storage.

Customized Protocol and Programmed Procedure for Automation Purification

To obtain a custom protocol for RNA size selection; or automating this procedure on a liquid handler or a magnetic processor, please contact AMD Biotech for instrument-specific instructions or additional processing procedures.

Phone: 404-290-5063 (in US), Email: support@amdbiotech.com

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via: Phone: 1-404-290-5063 (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.

Ordering Information

Product Description	Catalog No.	Size
Auto-Mag® RNA-Pure	S008-01 S008-02 S008-03 S008-04	5 ml 50 ml 250 ml 500 ml
Auto-Mag® RNA Elution Buffer	B232-01 B232-02 B232-03	50 ml 250 ml 500 ml

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