AMD Biotech Inc.
Advanced Molecular Detection

Product Manual

Auto-Mag® Plant Total RNA Isolation Kit

Version 2.0

Magnetic bead-based kit designed to extract high-quality total RNA from plant samples.

Catalog Number: R019-00, R019-01, R019-02,

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® Plant Total RNA Isolation Kit is specially designed for rapid and efficient purification of high-quality total RNA from a wide variety of plant species and tissue types. The kit utilizes Magnetic bead-based technology, and the standard procedure takes less than 30 minutes following cell lysis. The isolation can be performed in both 96-well plates (manually and automated) and in 1.5 ml tubes (manually only) with related protocols. Plant samples are disrupted in a homogenizer/bead-based milling equipment first. PRL Buffer is added to lyse the samples. The supernatant is then transferred to a new processing plate where Auto-Mag® C-5 beads are added to bind to the RNA. Following DNA are digested and a few wash steps, the highest integrity purified RNA is eluted and can be used in a wide range of downstream applications, such as Real-time PCR, Southern blotting, SNP analysis, NGS, hybridization applications, and other RNA-based analysis. The Auto-Mag® Plant Total RNA Isolation Kit can be adapted to high throughput liquid handling workstations.

Features:

- Fast and easy processing using a magnetic bead system
- Robust lysis system – chemical lysis combined with a mechanical homogenization
- Consistent, high yields of total RNA
- Isolate high-quality total RNA from a variety of plant species.
- Eluted RNA is well suitable for wide range of downstream applications

Kit Contents

Product Number	R019-00	R019-01	R019-02
Preparation	5	96	384
Auto-Mag® C-5	0.06 ml	1.1 ml	4.4 ml
PRL Buffer	3.5 ml	60 ml	240 ml
MRW Buffer *	2.25 ml	36 ml	144 ml
RW2 Buffer *	2 ml	40 ml	160 ml**
250ml Bottle for RW2 Prep.	-	-	1**
DNase I Digestion Buffer (1X)	0.8 ml	12 ml	48 ml
DNase I	0.015 ml	0.2 ml	0.8 ml
RNA Elution Buffer	1 ml	15 ml	60 ml
* Ethanol must be added prior to use. See Preparation of Reagents ** Please following the instructions at Preparation of Reagents.			

Storage and Stability

Auto-Mag® Plant Total RNA Isolation Kit is shipped at ambient temperature. All components are stable for 12 months when stored accordingly. Auto-Mag® C-5 should be stored at 2-8°C. DNase I must be stored at -20°C. All remaining components can be stored at room temperature (15-25°C).
During shipment or storage in cool ambient conditions, the precipitates may form in some buffers. Check buffers and re-dissolve any precipitates by warming the buffer at 37˚C. and gently shaking before using.

Preparation of Reagents

1. Prepare a fresh aliquot of PRL Buffer supplemented with DTT according to the following table. Prepare only what is needed and mix completely.

Reagent	Volume per test	Volume (ml)
PRL Buffer	600µl	1ml
2M DTT	12µl	20µl
Note: Only 600 µl of the Lysis Buffer with DTT is required for each sample disruption.		

2. Dilute MRW Buffer with 100% Ethanol as follows and store at room temperature.

Reagents	Kit	100% Ethanol to be Added
MPW Buffer	R019-00	1.5 ml
	R019-01	25 ml
	R019-02	95 ml
Please pay attention to prevent RNase contamination		

3. Dilute RW2 Buffer with 100% Ethanol as follows and store at room temperature.

Reagents	Kit	100% Ethanol to be Added
RW2 Buffer	R019-00	8 ml
	R019-01	200 ml
	R019-02	RW2 buffer diluting process for 384 package kit: 1. Alique 40ml of undiluted RW2 buffer into a 250ml empty bottle. 2. Add 200ml of 100% ethanol, close the lid tightly and mix thoroughly. 3. Reuse the bottle for the next RW2 buffer dilution
Please pay attention to prevent RNase contamination		

Additional Information

1. Specification

Features	Specification
Isolation Technology	Magnetic Beads
Sample Sources	Plant tissue, Seed, etc.
Starting Amount	Up to 50mg
Typical Yield	Dependent upon sample
Elution Volume	50-100 µl
Processing format	Auto, Manual
Downstream Application	RT-PCR, Northern blotting. Nuclease protection assay. RNA-Seq, NGS. etc.

2. Typical RNA yields from ~50 mg of some plant samples

Note: The RNA yield varies with the plant sample, RNA content of the sample, and age of the sample.

Plant sample Source	RNA yield*
Tomato leaves	10–40 µg
Green onion leaves	5–10 µg
Corn leaves	10–20 µg
Pine needles	2–15 µg
Lemon leaves	10–18 µg
Orange leaves	10-15 µg
Bell pepper leaves	15-25 µg
Spinach leaves	10–15 µg
*The RNA yield varies with the plant sample, RNA content of the sample, and age of the sample.	

3. 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® Plant Total RNA Isolation Protocol

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) from each product supplier.

Materials and Equipment to be supplied by User:

- 2.0 ml Homogenizer Bead Beating Tubes, or Prefilled Tissue Disruptor Tube (RNase free), (Cat. #: AMD B014)
- Equipment for disrupting plant tissue (Geno/Grinder, Mixer Mill, or Bead Mill Homogenizers etc.)
- 3-5 mm tungsten carbide beads
- Single-tube format: Nuclease-free 1.5 ml microcentrifuge tube
- 96-well format: 2 ml deep-well plate
- Sealing film
- Magnetic separation device
- Vortex
- Centrifuge capable of ≥16,000 × g for microcentrifuge tubes
- Centrifuge capable of 3000–4000 × g with swinging-bucket rotor for 96-well deep well plates
- Water bath, incubator, or heat block capable of 65°C
- Liquid nitrogen for freezing/disrupting samples
- 2M DTT
- 100% Ethanol

Before Starting

- Please to read this booklet in its entirety to become familiar with the procedures
- Prepare reagents according to instructions of Preparation Reagents on page 3.
- Preset water bath, incubator, or heating blocks to 65°C.
- Preheat Elution Buffer to 65°C.
- Suspension Auto-Mag® C-5 beads by vortex.

Protocol for Single-tube format

1. Disrupt the plant tissue：

(1) Disrupt the sample mechanically

Plant tissue can be homogenized with a rotor-stator, bead mill, or high-throughput homogenizers. We recommend bead mill or high-throughput homogenizers paired with stainless steel beads. When using beads for homogenization, ensure that the correct tubes are used, which typically have thicker walls and a screw cap with an O-ring.

- Place 30-50mg of fresh plant tissue or 10–20mg of seed samples into 2.0 ml Homogenizer Bead Beating Tubes in the presence of one or two grinding beads.

- Grind sample using a mechanical grinder following the manufacturer's instructions.
- After sample homogenization, go to step 2 immediately.

(2) Disrupt the sample manually:

- Place 30-50mg of fresh plant tissue or 10–20 mg of seed samples into a mortar that contains liquid nitrogen,
- Grind the tissue thoroughly using a clean pestle, then allow the liquid nitrogen to evaporate
- Transfer the powder to a Nuclease-free 1.5 ml microcentrifuge tube and go to step 2 immediately.

2. Add 600 µl of PRL Buffer with DTT to the sample tube, vortex at maximum speed for 20 seconds or pipette mix 20 times.

   Note: All ground material must be thoroughly mixed with the PRL Buffer (Plant RNA Lysis Buffer) as quickly as possible to avoid RNA degradation. Ground tissues can be used immediately in the RNA isolation procedure or stored at -80°C until use.

3. Incubate the sample tube at 65°C for 10 minutes. Occasionally mix the sample 2-3 times during incubation.

4. Centrifuge the sample tube at 16,000 x g at room temperature for 5 minutes to pellet the undigested debris. Collect and transfer ~400 µl of the supernatant to a new RNase-free 1.5-ml microcentrifuge tube.

   Note: Use centrifuge for microcentrifuge tubes. Do not transfer any pellet or other debris.

5. Add 200µl 100% Ethanol, 10µl Auto-Mag® C-5 to each sample, vortex at maximum speed for 20 seconds or pipette mix 20 times. Incubate the sample at room temperature for 5 minutes.

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   Note: Complete resuspension of the Auto-Mag® C-5 beads are crucial for obtaining purity.

6. Place the samples tube on a compatible magnetic separation device for 5 minutes or until Auto-Mag® C-5 beads are completely cleared from the solution. Remove and discard all the liquid. Do not disturb the attracted beads.

7. Remove the sample tube from the magnet. Add 600µl MRW Buffer and resuspend the Auto-Mag® C-5 beads by vortex at maximum speed for 20 seconds or pipette mix 20 times.

   Note: MRW Buffer must be diluted with ethanol prior to use. Complete resuspension of the magnetic beads is critical for obtaining high quality RNA.

8. Place the sample tube on the magnet for 2 minutes or until Auto-Mag® C-5 beads are completely cleared from the solution. Remove and discard all the liquid. Do not disturb the attracted beads.

9. Remove the sample tube from the magnet. Add 600µl RW2 Buffer and resuspend the Auto-Mag® C-5 beads by vortex at maximum speed for 20 seconds or pipette mix 20 times.

   Note: RW2 Buffer must be diluted with ethanol before use. Complete resuspension of the magnetic beads is critical for obtaining high quality RNA.

10. Place the sample tube on the magnet for 2 minutes or until Auto-Mag® C-5 beads are completely cleared from The solution. Remove and discard all the liquid. Do not disturb the attracted beads.

    Note: If total nucleic acid (RNA and DNA) is desired, skip 11-15, and proceed to step 16.

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

Note: All liquid must be aspirated at this step.

12. While the samples are drying, prepare the DNase I mixture. For each sample, gently mix 98µl of DNase I Digestion Buffer and 2µl DNase I.

13. Remove the sample tube from the magnet. Add 100µl DNase I mixture to each sample. Gently Mix by pipetting up and down 20 times to fully resuspend the magnetic beads. Incubate the samples tube at 37°C for 15 minutes.

Note: Avoid extensive vortex or pipetting as this may denature the DNase I.

14. Add 600µl RW2 Buffer to the sample for rebinding the RNA. Resuspend the Auto-Mag® C-5 beads by vortex at maximum speed for 20 seconds or pipette mix 20 times. Incubate samples at room temperature for 1 minute.

15. Place the sample tube on the magnet for 2 minutes or until Auto-Mag® C-5 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

16. Repeat Steps 14-15 for one more RW2 buffer wash.

17. Keep the sample tube 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 liquid from each tube.

18. Add 50~100µl RNA Elution Buffer to the sample and resuspend the Auto-Mag® C-5 beads by vortex for 20 seconds or pipette mix 20 times Incubate the sample at room temperature for 5-10 minutes.

Note: Incubate the sample tube at 65°C for 5 minutes to improve yield.

19. Place the sample tube back on the magnet for 5 minutes or until the Auto-Mag® C-5 beads are completely cleared from elution buffer.

20. Transfer the eluate (cleared supernatant) containing purified RNA to a nuclease-free storage vessel, and store purified RNA at -80 °C.

Protocol for 96-well format

1. Disrupt the plant tissue：

High-throughput homogenizers offer an appropriate method for handling 96 samples simultaneously. To prepare samples in 96-well plate format, place samples in a well of 96-well deep-well plate or capped microtube rack in the presence of one or two grinding beads.

- Place 30-50mg of fresh plant tissue or 10–20 mg of seed samples into well of 96-well deep-well plate, or in racked 96-well collection microtubes.
- Grind sample using a mechanical grinder following the manufacturer's instructions.
- After sample homogenization, go to step 2 immediately

2. Add 600 µl of PRL Buffer with DTT to each sample. Seal the plate with sealing film (not provided). Vortex to mix thoroughly to make sure that all clumps are dispersed.

   Note: All ground material must be thoroughly mixed with the PRL Buffer (Plant RNA Lysis Buffer) as quickly as possible to avoid RNA degradation. Ground tissues can be used immediately in the RNA isolation procedure or stored at -80°C until use.

3. Incubate the sample at 65°C for 10 minutes.

4. Centrifuge at maximum speed 4000×g for 10 minutes to pellet the undigested debris. Collect and transfer ~400µl of the supernatant to a new 96-well deep-well plates

   Note: Use Centrifuge with swinging-bucket rotor for 96-well deep well plates. Do not transfer any pellet or other debris.

5. Add 400µl 100% Ethanol, 10µl Auto-Mag® C-5 to each sample. Seal plate and vortex to mix thoroughly. Incubate the sample at room temperature for 5 minutes.

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   Note: Complete resuspension of the Auto-Mag® C-5 beads are crucial for obtaining purity.

6. Place the samples plate on a compatible magnetic separation device for 5 minutes or until Auto-Mag® C-5 beads are completely cleared from the solution. Remove and discard all the liquid. Do not disturb the attracted beads.

7. Remove the sample plate from the magnet. Add 600µl MRW Buffer. Seal the plate and vortex to mix thoroughly

   Note: MRW Buffer must be diluted with ethanol prior to use. Complete resuspension of the magnetic beads is critical for obtaining high quality RNA.

8. Place the samples plate on the magnet for 5 minutes or until Auto-Mag® C-5 beads are completely cleared from the supernatant. Remove and discard all the liquid. Do not disturb the attracted beads.

9. Remove the sample plate from the magnet. Add 600µl RW2 Buffer. Seal the plate and vortex to mix thoroughly.

   Note: RW2 Buffer must be diluted with ethanol before use. Complete resuspension of the magnetic beads is critical for obtaining high quality RNA.

10. Place the samples plate on the magnet for 5 minutes or until Auto-Mag® C-5 beads are completely cleared from the supernatant. Remove and discard all the liquid. Do not disturb the attracted beads

Note: If total nucleic acid (RNA and DNA) is desired, skip 11-15, and proceed to step 16.

11. 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: All liquid must be aspirated at this step.

12. While the samples are drying, prepare the DNase I mixture. For each sample, gently mix 98µl of DNase I Digestion Buffer and 2µl DNase I.

13. Add 100µl DNase I mixture to each sample. Gently Mix by pipetting up and down 20 times to fully resuspend the magnetic beads. Seal the plate and incubate at 37°C for 15 minutes.

Note: Avoid extensive vortex or pipetting as this may denature the DNase I.

14. Add 500µl RW2 Buffer to the sample for rebinding the RNA. Seal the plate and vortex to mix thoroughly. Incubate samples at room temperature for 2 minutes.

15. Place the sample plate on the magnet for 2 minutes or until Auto-Mag® C-5 beads are completely cleared from supernatant. Remove and discard all the liquid. Do not disturb the attracted beads.

16. Repeat Steps 14-15 for one more RW2 buffer wash.

17. 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 liquid from each tube.

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18. Add 50~100µl RNA Elution Buffer to the sample. Seal the plate and vortex to mix thoroughly. Incubate the sample at room temperature for 5-10 minutes.

Note: Incubate the sample tube at 65°C for 5 minutes to improve yield.

19. Place the sample plate back on the magnet for 5 minutes or until the Auto-Mag® C-5 beads are completely cleared from elution buffer.

20. Transfer the eluate (cleared supernatant) containing purified RNA to a nuclease-free storage vessel, and store purified RNA at -80 °C.

Protocol and Programmed procedure for Automation purification

Please contact technical support via: 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: 404-290-5063 (in US), Email: support@amdbiotech.com

Symptoms	Possible Causes	Comments
Low RNA yield	The sample was not stored properly, causing RNA degradation	Use fresh samples. Ensure that samples are processed immediately after collection or removal from storage. If immediate processing is not possible, flash freeze the samples in liquid nitrogen, then store at –80°C.
	The sample was not sufficiently homogenized.	To adequately disrupt the cell wall and therefore release the RNA, it is important to homogenize the sample thoroughly. Efficient homogenization and lysis of the plant cells increases the RNA yield.
	Ethanol was not added to the lysate.	Ensure the ethanol was added to the lysate to bind the RNA to Auto-Mag® C-5.
	The RNA rebinding step was not performed.	Ensure correct volume RWB Buffer are added after DNase l treatment.
	Beads were lost during purification.	Avoid disturbing the Auto-Mag® C-5 during aspiration of supernatant
	Ethanol is not added into MRW and RW2 Buffer	Add absolute 100% Ethanol to MRW and RW2 Buffer.
Auto-Mag® C-5 beads do not completely clear from solution	Too short of magnetizing time	Increase collection time on the magnet.
Problems in downstream applications	Ethanol carry-over	Dry the Auto-Mag® C-5 completely before elution.

Ordering Information

Product Description	Catalog No.	Size
Auto-Mag® Plant Total RNA Isolation Kit	R019-00 R019-01 R019-02 R019-Bulk	5 Preps. 96 Preps. 384 Preps. Request
Prefilled Tissue Disruptor Tube (RNase free)	B014-00 B014-01 B014-02 B014-Bulk	5 100 200 Request

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