Auto-Mag® Tissue DNA/RNA Co-Isolation Kit

Cat # AMD-DR03 Version 2.0

- ❖ Genomic DNA or Total RNA isolation from tissues, and cultured cells

Highlights

- Magnetic bead-based chemistry with no centrifugation or filtration
- One set of reagents and one protocol for DNA, RNA, or total nucleic acid isolation
- High yields even for a variety of sample types
- Complete removal of inhibitors
- High-quality DNA or RNA suitable for Downstream applications
- Optimized protocols for a range of starting materials

Protocol Contents

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

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Product Introduction

Auto-Mag® Tissue DNA/RNA Co-Isolation Kit is specially designed to use the special lysis condition with Auto-Mag® magnetic particles technology to isolate the high-quality total nucleic acid, DNA or RNA from 10-30mg of tissue or 1 x 10^6 of cultured cells. In contrast to conventional DNA or RNA extraction procedures that need two separate kits, AMD Auto-Mag® Tissue DNA/RNA Co-Isolation Kit use one set of reagents and one protocol to isolate simultaneously the total nucleic acid, genomic DNA or total RNA. Following the protocol, the purified DNA or RNA is suitable for all major downstream applications such as NGS, RNA-Seq, RT-PCR, and hybridization applications.

Application

DNA RNA isolation for:

- PCR, RT-PCR, Real-time PCR
- Cloning, genotyping
- Sequencing, NGS, or RNAseq.

Kit Contents

Product Number	AMD-DR03-10	AMD-DR03-50	AMD-DR03-200
Preparations	10	50	200
Auto-Mag® D-3	0.12 ml	0.55 ml	2.1 ml
TDRL Buffer	6 ml	28 ml	110 ml
DCE Buffer *	1.6 ml	8 ml	24 ml
DRW1 Buffer*	4.5 ml	22.5 ml	75 ml
DRW2 Buffer*	4 ml	20 ml	25 ml x 3
1X Digestion Buffer	1.5 ml	8 ml	25 ml
Auto-Mag® DNase I	0.025 ml	0.11 ml	0.42 ml
RNase A	0.025 ml	0.11 ml	0.42 ml
Proteinase K Solution	0.22 ml	1.1 ml	4.2 ml
RNA Elution Buffer	1.5 ml	8 ml	30 ml

* Ethanol must be added prior to use. See Preparation of Reagents

Storage and Stability

Auto-Mag® Tissue DNA/RNA Co-Isolation Kit is shipped at room temperature. However, after receiving the kit, please store the components in their appropriate conditions. All components of the Auto-Mag® Tissue DNA/RNA Co-Isolation Kit is guaranteed for at least 12 months from the date of purchase when the components of kit are stored as following: Auto-Mag® DNase I and RNase A must be stored at -20°C, Auto-Mag® D-3 should be stored at 2-8°C. Store all other components at room temperature (18-25°C). Check buffers for precipitates before use. Re-dissolve any precipitates by warming the product to 37˚C.

Preparation of Reagents

Prepare the following components for each kit before use:

- Dilute DCE Buffer, DRW1 and DRW2 with 100% ethanol as follows and store at room temperature.

	100% Ethanol to be added		
Kit	DCE Buffer	DRW1 Buffer	DRW2 Buffer
AMD-DR03-10	4 ml	4 ml	16 ml
AMD-DR03-50	20 ml	15 ml	80 ml
AMD-DR03-200	60 ml	50 ml	100 ml x3

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

Tissue DNA /RNA Co-Isolation Basic Protocol

Materials and Equipment to be supplied by User:

- 1.5 ml Nuclease-free micro-centrifuge tubes
- Refrigerated microcentrifuge
- Magnetic separation device
- Vortexer
- Equipment for disrupting and homogenizing tissue
- 100% Ethanol
- Water bath, incubator, or heat block capable of 65°C
- Pipette and nuclease-free pipette tips

Before Starting

- Ensure the work area is Nuclease free.
- Prepare reagents according to Preparation of Reagents Section.
- Preset water bath, incubator or heating blocks to 65°C.
- Completely resuspension Auto-Mag® D-3 by vortexing.

Procedure

1. Homogenize the tissue or cells samples using an appropriate method. See below for examples of common homogenization methods.

- For tissue samples:

  1). Mortar and Pestle: Collect 10-30mg fresh or preserved tissue sample in a mortar and freeze in liquid nitrogen. Grind the tissue using a clean pestle. Transfer the ground powder and liquid nitrogen into 1.5 ml Nuclease-free microcentrifuge tube and allow the liquid nitrogen to evaporate. Add 500µl TDRL Buffer and vortex at maximum speed for 1 minute or pipette mix 20 times. Incubate sample tube at room temperature for 10 minutes.

  2. Bead-beating: Add 10-30mg fresh or preserved tissue sample and 500µl TDRL Buffer into Pre-filled Tissue Homogenization Lysing Tubes with Grinding Beads. Grind the sample at a commercial bead-beater and then incubate sample at room temperature for 10 minutes. Parameters such as grinding speed, duration, temperature, and type of beads, etc. may need to be optimized. Refer to the manufacturer's manual for additional instructions

  Note: Proteinase K Solution is not necessary when using a mortar or bead-beating to grind tissue samples.

- For cultured cells:

  Resuspend the $1x10^6$ cultured cell pellet in 500µl TDRL Buffer by vortex at maximum speed for 1 minute or pipette mix 20 times. Add 20µl Proteinase K, mix immediately by vortex at maximum speed for 20 seconds

and incubate sample tube at room temperature for 10 min. Vortex briefly once during Incubation. If desired, using a rotor-stator homogenizer or passing through 20-gauge needle may increase yield.

2. Centrifuge the sample at 10,000 x *g* at 4°C for 10 minutes. Transfer 400µl clear lysate to a new 1.5 ml Nuclease-free tube. new tube. Do not disturb the debris pellet.

Note: If the clear lysate is less than 400µl, bring the volume up to 400µl with DRL2 Buffer. Or add same volume of diluted DCE at step3.

3. Add 400µl DCE buffer, and 10µl Auto-Mag® D-3 to each sample, vortex at maximum speed for 30 seconds or pipette mix 20 times. Incubate at room temperature for 10 minutes

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

4. Place the sample tube on the magnetic separation device and allow the magnetic beads to settle on the magnet for 5 minutes or until the supernatant turns completely clear. Remove and discard the cleared supernatant. Do not disturb the magnetic bead.

5. Remove the sample tube from the magnetic separation device. Add 600µl DRW1 Buffer to the sample and resuspend the magnetic beads by vortex at maximum speed for 30 seconds or pipette mix 20 times.

Note: DRW1 Buffer must be diluted with ethanol before use. Complete resuspension of the magnetic beads is critical for obtaining high quality DNA or RNA.

6. Place the sample tube back on the magnetic separation device and allow the magnetic beads to settle on the magnet for 5 minutes or until the supernatant turns completely clear. Remove and discard the cleared supernatant. Do not disturb the magnetic beads.

7. Remove the sample tube from the magnetic separation device. Add 600µl DRW2 Buffer to the sample and resuspend the magnetic beads by vortex at maximum speed for 30 seconds or pipette mix 20 times.

Note: DRW2 Buffer must be diluted with ethanol before use. Complete resuspension of the magnetic beads is critical for obtaining high quality DNA or RNA.

8. Place the sample tube back on the magnetic separation device and allow the magnetic beads to settle on the magnet for 5 minutes or until the supernatant turns completely clear. Remove and discard the cleared supernatant. Do not disturb the magnetic beads.

Note: All liquid must be aspirated at this step. It is advised to remove all liquid from the well first then wait one minute and use a fine pipet tip to remove any residual liquid.

9. Follow the steps in the table to isolation RNA or DNA or Total Nucleic Acids.

	A: For Total RNA only	B: For DNA only	C: For Total Nucleic Acids
10.	Leave the tube on the magnetic separation device for 5 minutes to air dry the magnetic beads.	Leave the tube on the magnetic separation device for 5 minutes to air dry the magnetic beads.	

11.	While the samples are drying, prepare the DNase I mixture. For each sample, gently mix 98µl of 1X Digestion Buffer and 2µl of Auto-Mag® DNase I.	While the samples are drying, prepare the RNase A mixture. For each sample, gently mix 98µl 1X Digestion Buffer and 2µl of RNase A.	Go to step 15
12.	Add 100µl DNase I mix to each sample. Mix by pipetting up and down to fully resuspend the magnetic beads. Incubate at room temperature for 10 minutes.	Add 100µl RNase A mix to each sample. Mix by pipetting up and down to fully Resuspend the magnetic beads. Incubate at room temperature for 10 minutes.	

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

13. Remove the sample tube from the magnetic separation device. Add 600µl DRW2 Buffer to the sample and resuspend the magnetic bead by vortex at maximum speed for 30 seconds or pipette mix 20 times. Incubate the sample tube at room temperature for 2 minutes.

Note: DRW2 Buffer must be diluted with ethanol before use. Complete resuspension of the magnetic beads is critical for obtaining high quality DNA or RNA.

14. Place the sample tube back on the magnetic separation device and allow the magnetic beads to settle on the magnet for 5 minutes or until the supernatant turns completely clear. Remove and discard the cleared supernatant. Do not disturb the magnetic beads.

15. Repeat Steps 13-14 for second wash.

16. Leave the sample tube on the magnetic separation device for 5 minutes to air dry the magnetic beads and remove any residual liquid with a fine pipet tip.

Note: It is critical to completely remove all liquid from each tube.

17. Add 50-100µl RNA Elution Buffer. Completely resuspend the magnetic beads by vortex at maximum speed for 30 seconds or pipette mix 20 times. Incubate for 10 minutes at room temperature.

Note: Incubate at 65°C may increase yield.

18. Place the tubes back on the magnetic separation device and wait 2-5 minutes or until the magnetic beads are completely cleared from Elution Buffer.

19. Transfer the eluate (cleared supernatant) containing purified RNA, or DNA, or total Nucleic acid to an appropriate storage vessel.

20. Store purified samples at properly conditions.

Sample	RNA	DNA	Total Nucleic acid
Storage condition	-80 °C	-20°C	-80°C

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 Recovery	Incomplete homogenization	Completely homogenize sample. Increase centrifugation time. Reduce the amount of the starting material.
	DNA or RNA remains on the Beads	Heat Elution Buffer to 65°C prior to elution and incubate sample tube at 65°C may increase yield.
	Sample is overloaded	Reduce the quantity of the starting material.
	Magnetic Beads	Make sure that the magnetic beads are being resuspended throughout bind, wash, and elution steps by shaking or pipet mixing the beads. A lack of proper mixing can yield poor results.
Nucleic acid degraded	RNase contamination	Ensure not to introduce RNase during the RNA Isolation procedure. Check buffers for RNase contamination.
	Sample input	To ensure sample stabilization and minimize degradation effects. Freeze tissue samples quickly in liquid nitrogen. Do not store tissue cultured cells prior to extraction. Follow protocol closely and work quickly. Check initial sample by establishing kit controls with a known quality and concentration to eliminate artifacts originating from kit to kit variation.
	Bead-beating	Bead-beating times may need to be optimized to ensure sufficient lysis without compromising sample quality. These exact settings can vary from low to high-speed cell disrupters. Bead-beating will shear genomic DNA to some extent.
RNA contamination at DNA isolation	Sample is overloaded	Reduce the amount of starting material. At step 12, digest with RNase A and incubate at 65°C for 5 minutes.
DNA contamination at RNA isolation	Sample is overloaded	Reduce the amount of starting material. At step 12, digest with RNase-free DNase 1 and incubate the sample tube at 37°C for 10 minutes.
Low Abs ratios	RNA diluted in acidic buffer or water	Nuclease-free water is slightly acidic and can lower A260/A280 ratios. Use TE buffer to dilute RNA prior to spectrophotometric analysis.
Downstream applications are unsuccessful	Presence of residual ethanol	Ensure to air dry the magnetic beads. Remove any residual liquid with a pipette before elution.
	Eluate contaminated with excess salt.	Ensure DRW2 Buffer has been diluted with 4 volumes of 100% ethanol as indicated on bottle. DRW2 Buffer must be stored and used at room temperature. Ensure that the wash in step 13 of the protocol is effective. Incubate the magnetic beads with the DRW2 Buffer for several minutes before proceeding to magnetic separation.