

MagBeads FastRNA Kit (Ready-to-Use for MPure-96)

Magnetic bead-based Purification for total DNA from tissue and
cell culture samples

Size: 96 preps

Storage: 15-25 °C

Cat. No.: 117034400

Content Version: Feb 2024



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1. Introduction to MagBeads FastRNA Kit

Magbeads FastRNA Kit is intended for simple and rapid extraction of total RNA from tissue and culture cells samples using the MPure-96™ aNAP System. The kit is based on superparamagnetic particles purification technology, no phenol-chloroform extraction or alcohol precipitation. Purified RNA is ready for downstream applications such as RT-PCR, virus RNA testing and so on. The kits buffers can be used for both manual extraction process and automatic nucleic acid extraction machines.

Magbeads FastRNA Kit combines the speed and efficiency of silica-based technology with the convenient handling of magnetic particles for purification of total RNA. Samples are lysed and RNA is purified from lysates in one step through its binding to the silica surface of the particles in the presence of a chaotropic salt. The particles are separated from the lysates using a magnet and DNA is removed by treatment with RNase-free DNase. The magnetic particles are efficiently washed, and RNA is eluted in RNase-free water.

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2. Kit Components and User Supplied Materials

2.1 MagBeads FastRNA Kit Component

MagBeads FastRNA Kit (#117034400, 96 Preps)	
Components	Package
Sample Plate	1 plate
Wash Plate 1	1 plate
Wash Plate 2	1 plate
Wash Plate 3	1 plate
Elution Plate	1 plate
DNase Plate	1 plate
Proteinase K	48 mg
Protease Dissolve Buffer	5 mL
DNase I	2 x 600µL
Buffer RTL	80 mL
Buffer MCB	30 mL
96 spin tips	1 ea

2.2 User Supplied Materials

- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Vortex mixer.
- Desktop microcentrifuge with rotor for 2 ml reaction tubes (RCF max. 16,000 x g).
- PCR box or Biological cabinet. • Vacuum aspirator with flask for removing supernatant.
- Tube racks.
- 1.5 ml polypropylene sterile tubes.
- Refrigerator for 2-8°C.
- Deep-freezer for ≤ -16°C.
- Waste bin for used tips.
- Permanent pen for labeling
- Thermostatic bath or dry block for tubes with controlled temperature and capable of incubating at 25-100°C.

3. Storage and Kit Stability

Magbeads Particles, DNase I and Proteinase K should be stored at 2 - 8 °C upon arrival. However, short-term storage (up to 12 weeks) at room temperature (15 - 25 °C) does not affect their performance. The remaining kit components can be stored dry at room temperature (15 - 25 °C) and are stable for at least 18 months under these conditions.

4. Important Consideration Before Use

- Add 2.4 mL Protease Dissolve Buffer into Proteinase K and store at -20 °C after it dissolves.
- Dilute Buffer MCB with isopropanol (ratio of Buffer MCB: Isopropanol = 3 : 7) and store at room temperature.
- (Optional) 2-mercaptoethanol can be added to an aliquot of RTL Lysis Buffer before use. Add 20µl 2-mercaptoethanol per 1 mL RTL Lysis Buffer. This mixture can be stored at room temperature for 2 weeks.

5. Safety Precautions

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiry date.
- Dispose of all samples and unused reagents in compliance with local authorities requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact with the skin, eyes and mucose membranes. If skin, eyes and mucose membranes contact immediately flush with water, seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one directional; it should begin in the Extraction Area move to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which the previous step was performed.

6. Protocol

Sample Preparation

A. Cell

Harvest cells (no more than 1×10^7 cells). For pelleted cells, loosen the cell pellet thoroughly by flicking the tube and add the appropriate volume of 500 μ L Buffer RTL. For direct lysis of cells grown in a monolayer, add 500 μ L Buffer RTL onto the cell-culture dish. Pass the lysate at least 5 times through a blunt 20-gauge needle fitted to an RNase-Free Syringe.

B. Animal Tissue

Do not use more than 20 mg of animal tissue. Disrupt and homogenize the sample, then add 600 μ L Buffer RTL. After lysis, centrifuge the lysate at 14,000 x g for 3 mins at room temperature.

C. Plant Tissue

Disrupt the plant sample using liquid nitrogen. Transfer up to 50 mg of powder to 1.5 mL microcentrifuge tube and add 600 μ L Buffer RTL. Vortex and centrifuge at 14,000 x g for 3 mins at room temperature.

D. Yeast Cell

Collect 5×10^6 yeast cells, then add 300 mg 0.4-0.6 g Glass Beads and 600 μ L RTL Lysis Buffer. Vortex at maximum speed for 10 mins. Centrifuge at 10,000 x g for 3 mins at room temperature.

E. Bacterial Cell

Collect 1×10^8 bacterial cells, then add 300 mg 0.1-0.26 g Glass Beads and 600 μ L RTL Lysis Buffer. Vortex at maximum speed for 10 mins. Centrifuge at 10,000 x g for 3 mins at room temperature.

MPure-96 Automation Purification Method

1. Transfer 500 μ L of supernatant and 20 μ L of Proteinase K carefully to sample plate containing Buffer MCB before place it at position 1 of Mpure-96™ aNAP System; Place the other reagent plates in the instrument according to the following table and run the instrument with program named “MagBead_RNA”:

Step	Position	Process	Time (s)			Spin (rpm)	Temp (C)
			Mix	Vapor	Collect		
1	2	MagBeads Preparation	0	0	30	0	RT
2	1	Bind	600	0	120	3000	RT
3	2	Wash 1	60	120	120	3000	RT
4	2	Dry	0	120	0	0	RT
5	3	DNase I digestion	900	0	150	3000	RT
6	3	Addition of MCB buffer	300	0	150	3000	RT
7	4	Wash 2	180	0	150	3000	RT
8	5	Wash 3	180	0	150	3000	RT
9	5	Dry	0	600	0	0	RT
10	6	Elute	300	0	300	3000	55

- Carefully add 450 μ L Buffer MCB to the sample plate during dispense step and place it back to the instrument to continue the rest of the protocol.
- Transfer clear purified RNA to a clean 96-well microplate (not provided). The eluent is now ready for downstream applications. Store purified RNA at -80°C for extended periods.
Note: If there are still Magnetic Beads remaining in eluent, please centrifuge at 14,000 x g for 3-5 mins and take the supernatant again.

7. Troubleshooting

This guide may be useful in solving any problems that may arise. For further assistance, please contact our technical support team at apac-techsupport@mpbio.com

Problem	Recommendation
False negatives with extraction product	Degradation of the nucleic acid contained in the sample. Use a new sample, store samples appropriately.
	Loss of nucleic acid deposit. Carefully draw off the wash solution and try not to remove the nucleic acid deposit.
	Degradation of the extracted nucleic acid. Plastic free from DNAses and RNAses should be used. Use a new aliquot of kit's component.
False positives with extraction product	Contamination during sample extraction. One test tube at a time should be opened. Avoid spilling the contents of the test tube, always change tips.
	Contamination of the reagents prepared for the step. Use a new aliquot of a component.
	Contamination of the extraction zone by amplicons. Surfaces and instruments using aqueous detergents should be cleaned, wash lab coats, replace test tubes and tips in use.

8. Product Use Limitation & Warranty

The products presented in this instruction manual are for research or manufacturing use only. They are not to be used as drugs or medical devices to diagnose, cure, mitigate, treat, or prevent diseases in humans or animals, either as part of an accepted course of therapy or in experimental clinical investigation. These products are not to be used as food, food additives or general household items. Purchase of MP Biomedicals products does not grant rights to reproduce, modify, or repackage the products or any derivative thereof to third parties. MP Biomedicals makes no warranty of any kind, expressed or implied, including merchantability or fitness for any particular purpose, except that the products sold will meet our specifications at the time of delivery.

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