Instruction Manual
Research Use Only

MagBeads FastRNA Kit for FFPE

Magnetic bead-based Purification for total RNA from FFPE samples.

Size: 192 preps
Storage: 15-25 °C
Cat. No.: 116573192
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1. Introduction to MagBeads FastRNA Kit for FFPE

MagBeads FastRNA Kit for FFPE is intended for rapid extraction of RNA from tissue, cells, blood, and other clinical samples. RNA can be used directly for RT-PCR, quantitative RT-PCR and so on.

MagBeads FastRNA Kit for FFPE is based on the purification method of high binding magnetic particles. The sample is lysed and digested. RNA is released into the lysate. After addition of magnetic particles and binding solution, RNA will be adsorbed on the surface of magnetic particles, and impurities such as proteins will be removed without adsorption. The adsorbed particles were washed with washing buffer to remove the proteins and impurities, washed with ethanol to remove salts, and finally the RNA was eluted with Elution Buffer.

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2. Kit Components and User Supplied Materials

2.1 MagBeads FastRNA Kit for FFPE Component

MagBeads FastRNA Kit for FFPE (#116573192, 192 Preps)	
Components	Package
Magbeads Particles	4.5 mL
Proteinase K	96 mg
Protease Dissolve Buffer Glycerol/Tris/$CaCl_2$	6 mL
DNase I	4 x 600 µL
DNase Buffer Tris/MgCl2	120 mL
Buffer DPS Alkane mixture	200 mL
Buffer FRL Tris/EDTA/SDS	60 mL
Buffer AL Guanidine Salt	60 mL
Buffer MW1 Guanidine Salt	100 mL
Buffer MW2 Tris/NaCl	2 x 50 mL
Nuclease Free Water DEPC-Treated Water	60 mL

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

Proteinase K, DNase I, Magbeads Particles should be stored at 2-8°C upon arrival. However, short-term storage (up to 24 weeks) at room temperature (15-25°C) does not affect their performance. The remaining kit components can be stored at room temperature (15-25°C) and are stable for at least 18 months under these conditions.

4. Important Consideration Before Use

- ❑ Dilute Buffer MW1/MW2 with absolute ethanol and store at room temperature.
- ❑ Add 5 mL Protease Dissolve Buffer to the Proteinase K

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

1. Using a scalpel, trim off the excess paraffin on the sample block. Cut up to 8 sections (5~20 μm) and immediately transfer samples into a 2 mL microcentrifuge tube or a 2 mL 96-well sample plate (not provided).

Note: Discard the first 2 to 3 sections of sample if it has been exposed to air.

2. Add 600 μL Buffer DPS into the sample tube. Vortex the tube for 5s and briefly centrifuge to bring down the sample.
3. Incubate at 56°C for 3 to 5 mins and vortex for 5s to dissolve the paraffin.

Note: The Buffer DPS may turn opaque or cloudy. If this occurs, add additional Buffer DPS and repeat Step 2.

4. Centrifuge at 14,000 x g for 2 mins and carefully discard the supernatant without disturbing the pellet.
5. Add 150 μL Buffer FRL and 20 μL Proteinase K into the sample and vortex. Incubate at 55℃ for 15 mins. then incubate at 80℃ for 15 mins. Briefly centrifuge the sample and proceed to extraction step.

Note: Incubation at 80℃ can reverse the nucleic acids modified by formaldehyde. Prolonged incubation time will cause degradation of RNA.

Manual Extraction

1. Add 20 μL Magbeads Particles and 300 μL Isopropanol to the well of 96 deep-well Plate. Pipette mix 10 times and shake at 700~900 rpm for 6 mins. Place the deep-well plate on a magnetic plate and allow the beads to aggregate for 5 mins. With the plate on the magnetic stand, perform the aspiration, and discard the supernatant from the plate.
2. Add 500 μL Buffer MW1 and shake at 900~1200 rpm for 1 min to resuspend the beads. Place the tube on the magnetic rack for 1 min, then remove the supernatant. Leave the plate on the magnetic separation device. Wait 1 minute. Remove residual liquid with a pipettor. Dry the Magbeads particles for an additional 1 min.
3. Add 100 μL DNase Mixture (100 μL DNase Buffer + 10 μL DNase I) to the sample. Mix by

shaking at 600~900rpm for 10~15 mins.

4. Add 600 µL Buffer MW1 to the sample, shake for 5 mins. Place the tube on the magnetic rack for 1 min, then remove the supernatant.
5. Add 500 µL Buffer MW2 and shake for 1 min to resuspend the beads. Place the tube on the magnetic rack for 1 min, then remove the supernatant.
6. Repeat step 5 once.
7. Leave the plate on the magnetic separation device. Wait 1 min and remove the residual liquid with a pipettor. Dry the Magbeads particles for additional 3-5 mins.
8. Add 30~100 µL RNase Free Water to the sample and mix by shaking for 5 mins. Place the tube on the magnetic rack for 3 mins.
9. Transfer the clear supernatant containing purified RNA to a new tube, and store the RNA at -20°C.

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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