Instruction Manual
Research Use Only

MagBeads FastDNA Kit for FFPE

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

Size: 96 preps
Storage: 15-25 °C
Cat. No.: 116576096
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Table of Contents

1. Introduction to MagBeads FastDNA Kit for FFPE

MagBeads FastDNA® Kit for FFPE is intended for rapid extraction of DNA from FFPE sample. This kit uses two combination methods. High-salt Bind is conducive to remove pigments or polysaccharides from complex FFPE samples, so as to improve the purity of nucleic acid and avoid blocking aligent 2100. Alcohol mediated adsorption is conducive for improving the nucleic acid yield.

MagBeads FastDNA® Kit for FFPE is based on the purification method of high binding magnetic particles. The sample is lysed and digested. DNA is released into the lysate. After addition of magnetic particles and binding solution, DNA 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 DNA was eluted with Elution Buffer.

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2. Kit Components and User Supplied Materials

2.1 MagBeads FastDNA Kit for FFPE Component

MagBeads FastDNA Kit for FFPE (#116576096, 96 Preps)	
Components	Package
Magbeads Particles	2.5 mL
Proteinase K	48 mg
Protease Dissolve Buffer Glycerol/Tris/$CaCl_2$	6 mL
RNase A Ribonuclease	20 mg
Buffer DPS Alkane mixture	100 mL
Buffer ATL Tris/EDTA/SDS	30 mL
Buffer BST1 Guanidine Salt	60 mL
Buffer BW1 Guanidine Salt	44 mL
Elution Buffer Tris/EDTA	30 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, RNase A, 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

- ❑ Add 2.4ml Protease Dissolve Buffer into the Proteinase K, and store at -20~8℃ after it dissolves.
- ❑ Add 1.3ml Protease Dissolve Buffer to the RNase, and store at -20~8℃ after it dissolves.
- ❑ Add 56 ml absolute ethanol to buffer BW1 and store at room temperature.

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 excess paraffin off the sample block. Cut sections 10 - 20 μm thick. Transfer 1- 5 sections to 1.5ml microcentrifuge tube. Add 600μl Buffer DPS (Deparaffinization Solution) to the sample. Vortex for 5s and centrifuge briefly to bring the sample to the bottom of the tube.
2. Incubate at 56˚C for 5 min and vortex vigorously for 15 s to dissolve the paraffin completely. If too little Buffer DPS is used or if too much paraffin is carried over with the sample, the Buffer DPS may become waxy or solid after cooling. If this occurs, add additional Buffer DPS and repeat the 56˚C incubation.
3. Centrifuge at full speed for 1 min to spin down any FFPE tissue that sticks to the tube wall or under the cap of the tube after vortexing. When the sample is sufficient, the dewaxing liquid can be aspirated and discarded to facilitate operation.
4. Add 200μl Buffer ATL into the bottom of tube.
5. Add 20μl proteinase K to the lower phase. Mix gently by pipetting up and down.
6. Incubate at 56˚C for 60 min (or until the sample has been completely lysed), then 90˚C for 60 min.
7. Briefly centrifuge the tube and transfer the lower phase into a new microcentrifuge tube.

Manual Protocol - Hight Salt Bind

1. Add 20μl Magbeads Particles N and 400μl Buffer BST1 to the lysate from step 7 (Sample Preparation). Mix thoroughly by vortex for 10~15seconds.
2. Incubate for 3 minutes and mix occasionally. Place the tube on the magnetic stand for 2 minutes until the beads have formed a tight pellet. Then remove the supernatant.
3. Add 500μl Buffer BW1 and vortex for 15 seconds to resuspend the beads. Place the tube on the magnetic stand for 2 minutes until the beads have formed a tight pellet. Then remove the supernatant.
4. Add 500μl 75% ethanol, and vortex for 15 seconds to resuspend the beads. Place the

tube on the magnetic stand for 1 minute until the beads have formed a tight pellet. Then remove the supernatant.

5. Add 500µl 75% ethanol, and vortex for 15 seconds to resuspend the beads. Place the tube on the magnetic stand for 1 minute until the beads have formed a tight pellet. Then remove the supernatant
6. Centrifuge briefly to collect the liquid on the tube and remove all the liquid. Air dry for 10 minutes.
7. Add 30~100µl Elution Buffer to the sample, re-suspend the beads by vortex. Incubate at 55°C for 10 minutes with shaking. If there is no shaking device, vortex 2~3 times to mix.
8. Place the tube on the magnetic rack for 3 minutes. Transfer the supernatant containing the purified DNA to a clean 1.5ml centrifuge tube.

Manual Protocol - Ethanol Bind

1. Add 20µl Magbeads Particles N and 200µl Buffer BST1 to the lysate from step 7 (Sample Preparation). Mix thoroughly by vortex from 10seconds.
2. Add 300µl absolute ethanol to the sample, mix thoroughly by inverting for 15~30 times. Incubate for 3 minutes and mix occasionally. Place the tube on the magnetic stand for 1 minute until the beads have formed a tight pellet. Then remove the supernatant.
3. Add 500µl Buffer BW1 and vortex for 15 seconds to resuspend the beads. Place the tube on the magnetic stand for 2 minutes until the beads have formed a tight pellet. Then remove the supernatant.
4. Add 500µl 75% ethanol, and vortex for 15 seconds to resuspend the beads. Place the tube on the magnetic stand for 1 minute until the beads have formed a tight pellet. Then remove the supernatant.
5. Add 500µl 75% ethanol, and vortex for 15 seconds to resuspend the beads. Place the tube on the magnetic stand for 1 minute until the beads have formed a tight pellet. Then remove the supernatant.
6. Centrifuge briefly to collect the liquid on the tube and remove all the liquid. Air dry for 10 minutes.
7. Add 30~100µl Elution Buffer to the sample, re-suspend the beads by vortex. Incubate at 55°C for 10 minutes with shaking. If there is no shaking device, vortex 2~3 times to mix.

8. Place the tube on the magnetic rack for 3 minutes. Transfer the supernatant containing the purified DNA to a clean 1.5ml centrifuge tube.

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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