【Product Name】MagPure FFPE DNA Kit

【Product specifications】48 Preps/Kit, 96Preps/Kit

【Intended Use】

This product is suitable for rapid extraction of DNA from FFPE sample, tissue, cells, blood, swabs, blood spots, semen and other clinical samples. This product using size selection magnetic beads, which can selectively remove small sizes of DNA (100 ~ 300bp) from FFPE samples by adjusting the amount of binding solution, so as to improve the effective data volume of downstream NGS.

【Principle】

The sample is lysed and digested under the action of lysate and Protease. DNA is released into the lysate. After adding 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 solution to remove proteins and impurities, washed with ethanol to remove salts, and finally DNA was eluted by Elution Buffer.

【Main Composition】

Cat.No.	D632301B	D632302B	Composition
Preps	48 Preps	96 Preps	-
MagBind Particles	1.1 ml	2 x 1.1 ml	Magnetic Particles
RNase A	10 mg	20 mg	Ribonuclease
Proteinase K	24 mg	48 mg	Protease
Protease Dissolve Buffer	3 ml	6 ml	Glycorel/Tris/$CaCl_2$
Buffer DPS	60 ml	100 ml	alkane mixture
Buffer ATL	15 ml	30 ml	Tris/EDTA/SDS
Buffer AL	15 ml	30 ml	Guanidine Salt
Buffer BD*	6 ml	15 ml	sodium perchlorate
Buffer BXW1*	13 ml	44 ml	Guanidine Salt
Elution Buffer	15 ml	30 ml	Tris/EDTA

【Storage conditions and Validity】

RNase A, Proteinase K and MagBind Particles 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 at room temperature (15–25°C) and are stable for at least 18 months under these conditions.

【Preparation before Use】

- Add 1.2ml (48Preps) or 2.4ml(96 Preps) Protease Dissolve Buffer to the Proteinase K, and store at -20~8℃ after dissolve
- Add 0.6ml (48Preps) or 1.3ml(96 Preps) Protease Dissolve Buffer to the RNase, and store at -20~8℃ after dissolve.
- Add 24ml (48Preps) or 60 ml (96 Preps) absolute ethanol to buffer BD and store at room temperature
- Add 17ml (48Preps) or 56 ml (96 Preps) absolute ethanol to buffer BXW1 and store at room temperature

【Section A: Sample Prepare】

A . FFPE Samples

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.
4. Add 150 µl Buffer ATL to the bottom of tube.
5. Add 20 µl 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.

   The incubation at 90°C in Buffer ATL partially reverses formaldehyde modification of nucleic acids. Longer incubation times or higher incubation temperatures may result in more fragmented DNA. If using only one heating block, leave the sample at room temperature after the 56°C incubation until the heating block has reached 90°C.
7. Briefly centrifuge the tube and transfer the lower phase into a new microcentrifuge tube.
8. Add 10µl RNase A to the sample and mix well. Incubate for 10min at room temperature.
9. Add 150µl Buffer AL and mix well by vortexing. Proceed section B or Section C.

B: Solid tissue (1~10mg)

1. Cut ~10mg tissue into small pieces and transfer into a new 1.5ml centrifuge tube.

2. Add 150µl Buffer ATL and 20ul Proteinase K to the sample, mix well.
3. Incubate at 56°C for 60 min (or until the sample has been completely lysed.
4. Add 10 µl RNase A to the sample and mix well. Incubate for 10min at room temperature.
5. Add 150µl Buffer AL to the samples, vortex to mix and incubation at 70°C for 10 minutes.
6. Proceed section B or Section C.

C. Anticoagulated blood or Plasma (150uL)

1. Transfer 20ul Proteinase K to a new 1.5ml centrifuge tube.
2. Add150µl whole blood, plasma or other body fluids to the tube.
3. Add 150µl Buffer AL and mix well. Incubate at 70°C for 10 minutes.
4. Proceed section B or Section C.

D. Blood stains/Seminal Spots

1. Transfer the 1 slices(3mm) to the 1.5ml centrifuge tube. Add 150 µl Buffer ATL and 20 µl Proteinase K to the sample. Incubate at 56°C for 60 min with shaking.
2. Add 150 µl Buffer AL and incbuate for 10 min at 70°C with shaking.
3. Centrifuge at 13,000 x g for 1 min and Transfer 300ul of the supernatant to a new centrifuge tube.
4. Proceed section B or Section C.

E. Swab

1. Transfer 1 Swab to the 2ml centrifuge tube. Add 350~500µl Buffer ATL and 20µl Proteinase K to the sample. Incubate at 56°C for 20~30 min with shaking.
2. Centrifuge at 13,000 x g for 1 min.
3. Transfer 300ul of the lysate to a new centrifuge tube. Proceed section B or Section C.

Section B: Manul Protocol

1. **Add 20µl MagBind Particles and 300~400µl Buffer BD** to the samples or the supernatant. Mix thoroughly by inverting for 15~30 times. Incubate for 6 minutes and mix occasionally. Place the tube to the magnetic stand for 5 minutes until the beads have formed a tight pellet. Then remove the supernatant.

   When processing FFPE samples, the amount of buffer BD can be adjusted to selectively remove 100-300bp fragments. 300ul buffer BD can remove fragments below 500bp; 400ul buffer BD can remove 200bp fragment. When processing tissue, cell, blood and other samples, add 300ul buffer BD.

2. **Add 500µl Buffer BXW1** and vortex for 15 seconds to re-suspend beads. Place the tube to the magnetic stand for 2 minute until the beads have form a tight pellet. Then remove the supernatant.
3. **Add 500µl 75% ethaonl,** and vortex for 15 seconds to re-suspend beads. Place the tube to the magnetic stand for 1 minute until the beads have form a tight pellet. Then remove the supernatant.
4. **Add 500µl 75% ethaonl,** and vortex for 15 seconds to re-suspend beads. Place the tube to the magnetic stand for 1 minute until the beads have form a tight pellet. Then remove the supernatant.
5. Centrifuge shortly to collect liquid on the tube and remove all the liquid. Air dry for 3 minutes.
6. **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 .
7. Place the tube to the magnetic rack for 3 minutes.
8. Transfer the supernatant containing the purified DNA to a clean 1.5ml centrifuge tube.

Section C: Auto Purify by KingFisher Flex

1. Add the Reagents/sample to the wells of the deep well plate according to the table below.

Name of the Plate	Pre-loaded reagents	Addition before use
Sample plate	300~400µl Buffer BD 20µl MagBind Particle	300µl Lysate or suprnatant
	When processing FFPE samples, the amount of buffer BD can be adjusted to selectively remove 100-300bp fragments. 300ul buffer BD can remove fragments below 500bp; 400ul buffer BD can remove 200bp fragment. When processing tissue, cell, blood and other samples, add 300ul buffer BD.	
Wash Plate 1	500µl Buffer BXW1, Put in 96 magnetic Tip	
Wash Plate 2	500µl 75% ethanol	
Wash Plate 3	500µl 75% ethanol	
Elution plate	40-100µl Elution Buffer	

2. Turn on the machine, start the corresponding program(D6323B).
3. Place the 96-well plate into the instrument as prompted.
4. Finish the operation after ~30 minutes.
5. Remove the 96-well plate and magnetic jacket.
6. Store the Eluted product at -20~8℃.