

[Product Name] MagPure FFPE DNA/RNA Kit

[Product specifications] 48 Preps/Kit, 200 Preps/Kit

【Intended Use】

The Kit is specially designed for simultaneous purification of genomic DNA and total RNA from formalin-fixed, paraffin-embedded (FFPE) tissue sections. Purified DNA/RNA are suitable for use in applications such as real-time PCR and Pyrosequencing.

[Principle]

FFPE samples are incubated in an optimized lysis buffer, which results in the release of RNA and precipitation of DNA. After centrifugation, the RNA-containing supernatant and DNA-containing pellet are then processed separately to purify RNA and DNA. After adding magnetic particles and binding solution, DNA/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 solution to remove proteins and impurities, washed with ethanol to remove salts, and finally DNA/RNA was eluted by RNase Free Water.

[Main Composition]

Cat.No.	IVD3026-48	IVD3026	Composition
Purification Times	48 Preps	200 Preps	-
MagBind Particles	2 x 1.1 ml	9.0 ml	Magnetic Particles
Proteinase K	50 mg	180 mg	Protease
Protease Dissolve Buffer	3 ml	10 ml	$Glycorel/Tris/CaCl_2$
Buffer DPS	50 ml	150 ml	alkane mixture
Buffer FRL	10 ml	40 ml	Tris/EDTA/SDS
Buffer ATL	10 ml	40 ml	Tris/EDTA/SDS
Buffer AL	20 ml	80 ml	Guanidine Salt
Buffer BXVV1 *	44 ml	110 ml	Guanidine Salt
RNase Free Water	15 ml	30 ml	Tris/EDTA

[Storage conditions and Validity]

Proteinase K and MagBind 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.

[Preparation before Use]

- Add 2.5ml (48 Preps) or 9ml (200 Preps) Protease Dissolve Buffer to the bottle of Proteinase K, and store at -20~8℃ after dissolve.
- Add 56ml (48 Preps) or 140ml (200 Preps) absolute ethanol to buffer BXW1 and store at room temperature.

Protocol

- 1. Using a scalpel, trim excess paraffin off the sample block. Cut sections 10–20 µm thick.
- Transfer 1-6 sections to 1.5ml microcentrifuge tube. Add 700µl Buffer DPS (Deparaffinization Solution) to the sample. Vortex for 5s and centrifuge briefly to bring the sample to the bottom of the tube.

Do not use more than six 10 µm sections of 150 mm2 surface area or three 20 µm sections of 150 mm2 surface area. If the sample surface has been exposed to air, discard the first 2–3 sections.

- Incubate at 56°C for 5 min and vortex vigorously for 15 s to dissolve the paraffin completely.
 Centrifuge at full speed for 2 min. Remove the supernatant by pipetting without disturbing the pellet.
- 4. Centrifuge at 14,000 x g for 2 minutes. Aspirate and discard the supernatant carefully, do not disturb the pellet.
- 5. Resuspend the pellet by adding 150µl Buffer FRL and flicking the tube to loosen the pellet.
- 6. Add 20µl proteinase K and mix by vortexing. Incubate at 56°C for 15~30 min.
- 7. Incubate on ice for 3 min and centrifuge for 15 min at 20,000 x g.
- 8. Carefully transfer the supernatant, without disturbing the pellet, to a new1.5 ml microcentrifuge tube for RNA purification in steps 9–17. Keep the pellet for DNA purification in steps 18–27.

RNA Isolation

- 9. Incubate the supernatant at 80°C for 30 minutes.
- 10. Add 150µl Buffer AL to the sample and mix thoroughly.

- 11. Add 300µl Isopropanol and 20µl MagBind Particles to the sample. Mix thoroughly and incubate for 6 minutes, mix occasionally during incubation. Place the tube to the magnetic stand for 5 minutes until the beads have formed a tight pellet. Then remove the supernatant.
- Add 500µl Buffer BXW1 and vortex for 10 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.
- 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.
- Add 500µl 75% ethaonl, and vortex for 10 seconds to resuspend beads. Place the tube to the magnetic stand for 1 minute until the beads have form a tight pellet. Then remove the supernatant.
- 15. Centrifuge shortly to collect liquid on the tube and remove all the liquid. Air dry for 3 minutes.
- 16. Add 30~50µl RNase Free Water to the sample, re-suspend the beads by vortex. Incubate at room temperature for 5 minutes with shaking. If there is no shaking device, vortex 2~3 times to mix.
- Place the tube to the magnetic rack for 3 minutes. Transfer the supernatant containing the purified RNA to a clean 1.5ml centrifuge tube

DNA Isolation

- 18. Resuspend the pellet from step 8 in 150µl Buffer ATL and add 20µl proteinase K. Mix by vortexing.
- 19. Incubate at 56°C for 1 h. Incubate at 90°C for 1 h.
- 20. Add 150 µl Buffer AL and mix thoroughly by vortexing.
- 21. Add 300 µl Isopropanol and 20 µl MagBind Particles to the sample. 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.
- Add 500 µl 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.
- 23. Add 500 µl 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.

- 24. Add 500 µl 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.
- 25. Centrifuge shortly to collect liquid on the tube and remove all the liquid. Air dry for 3 minutes.
- 26. Add 30~50 µl RNase Free Water 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. Place the tube to the magnetic rack for 3 minutes.
- 27. Transfer the supernatant containing the purified DNA to a clean 1.5ml centrifuge tube

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	Reagent
Sample plate	300µl Isopropanol, 150µl Buffer AL, 20µl MagBind Particles 150µl Sample Lysate from step 9(RNA) or step 19(DNA).
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	40-80µl RNase Free Water

- 2. Turn on the machine, start the corresponding program.
- 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°C.