【Product Name】 MagPure FFPE RNA Kit

【Product specifications】20Preps/Kit, 200 Preps/Kit

【Intended Use】

This product is suitable for rapid extraction of RNA from FFPE tissue. RNA can be used directly for RT-PCR, quantitative RT-PCR and so on.

【Principle】

This product is based on the purification method of high binding magnetic particles. The sample is lysed and digested under the action of lysate and Protease. RNA is released into the lysate. After adding 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 solution to remove proteins and impurities, washed with ethanol to remove salts, and finally RNA was eluted by Buffer.

【Main Composition】

Cat.No.	IVD3022-20	IVD3022	Main Composition
Purification Times	20 Preps	200 Preps	-
MagBind Particles	0.5 ml	4.5 ml	Magnetic Particles
Proteinase K	12 mg	100 mg	Protease
Protease Dissolve Bffer	1.8 ml	6 ml	Glycorel/Tris/CaCl2
DNase I	600 µl	4 x 600 µl	DNase I
DNase Buffer	6 ml	30 ml	Tris/MgCl2
Buffer DPS	15 ml	200 ml	alkane mixture
Buffer FRL	5 ml	40 ml	Tris/EDTA/SDS
Buffer AL	5 ml	40 ml	Guanidine Salt
Buffer MW1*	13 ml	110 ml	Guanidine Salt
Buffer MW2*	6 ml	2 x 50 ml	Tris/NaCl
RNase Free Water	5 ml	30 ml	DEPC-Treated Water

【Storage conditions and Validity】

Proteinase K and MagBind Particles should be stored at 2–8°C upon arrival. DNase I should be stored at -20°C. However, short-term storage (DNase I up to 1 weeks, Proteinase K and MagBind Particles up to 8 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.

【Materials and Equipment to be Supplied by User】

- Add 17ml (20 Preps) or 140ml (250 Preps) 100% ethanol to the bottle of MW1 and store at room temperature.
- Add 24ml (20 Preps) or 2 x 200ml (250 Preps) 100% ethanol to the bottle of MW2 and store at room temperature.
- Add 0.6ml (20 Preps) or 5ml (200Preps) Protease Dissolve Buffer to the Proteinase K and store at -20~8℃ after dissolve.

A: Manual or Liquid Station Protocol:

1. Using a scalpel, trim excess paraffin off the sample block. **Cut up to 8 sections 5~20µm thick and immediately place the sections in a 1.5 or 2 ml microcentrifuge tube.**
   If the sample surface has been exposed to air, discard the first 2–3 sections,

2. **Add 0.6ml Buffer DPS into the sample and vortex for 5 seconds to mix thoroughly**. Centrifuge briefly to bring the sample to the bottom of the tube. Incubate at 56°C for 3~5 min and vortex for 5 seconds to dissolve paraffin.
   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 14,000 x g for 2 minutes. Aspirate and discard the supernatant carefully, do not disturb the pellet.

4. **Add 150µl Buffer FRL and 20µl Proteinase K to the sample and vortex. Incubate at 55℃ for 15 ~30 minutes then Incubate at 80℃ for 15 minutes.**
   Incubation at 80℃ can reverse the nucleic acids modified by formaldehyde. Prolonged incubation

time will cause degradation of RNA.

5. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid. **Add 150µl Buffer AL to the sample, vortex for 5 seconds.**

6. **Add 20µl MagBind Particles and 300µl Isopropanol to the well of 96 well Plate(2.2ml).** Pipette mix 10 times and then shaking at 700~900rpm for 6 minutes.Place the deep well plate on an Magnet Plate and allow beads to separate for 5 minutes. With the plate on the Magnet Plate, perform the aspiration, and then discard the supernatant from the plate.

7. **Add 500µl Buffer MW1 and shaking at 900~1200rpm for 1 minute to resuspend the particles.** Place the tube to the magnetic rack for 1 minute, then remove the supernatant. Leave the plate on the magnetic separation device. Wait 1 minute. Remove residual liquid with a pipettor. Dry the Mag-Pure Particles for an additional 1 minutes.

8. **Add 100µl DNase Mixture (100µl DNase Buffer + 10µl DNase I) to the sample.** Mix by shaking at 600~900rpm for10~15 minutes. **Add 600µl Buffer MW1 to the sample, shaking for 5 minutes.** Place the tube to the magnetic rack for 1 minutes, then remove the supernatant.

9. **Add 500µl Buffer MW2 and shaking for 1 minute to resuspend the particles.** Place the tube on the magnetic rack for 1 minutes, then remove the supernatant.

10. Repeat step 9 once.

11. Leave the plate on the magnetic separation device. Wait 1 minute and remove residual liquid with a pipettor. Dry the Mag-Pure Particles for an additional 3-5 minutes.

12. **Add 30~100µl RNase Free Water to sample and mix by shaking for 5 minutes.**Place the tube to the magnetic rack for 3 minutes.

13. Transfer the cleared supernatant containing purified RNA to a new Tube) and store RNA at -20°C.

B: Auto Purify by KingFisher Flex or similar Extractor isolation:

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

Name of the Plate	Pre-loaded reagents
Sample plate	150µl Lysate, 150µl Buffer AL and 300µl Isopropanol 20µl MagBind Particle
Wash Plate 1	500µl Buffer MW1, Put in 96 magnetic Tip
DNase	100µl DNase Buffer and 10µl DNase I
Wash Plate 2	500µl Buffer MW2
Wash Plate 3	500µl Buffer MW2
Elution plate	50µl RNase Free Water

2. Place a 96 tip comb for deep well magnets on Wash Plate 1.

3. Start the protocol with the KingFisher Flex and load the plates.

4. **Add 600µl Buffer MW1 to the DNase plate during the dispense step.**

5. Place the DNase plate back into the instrument and press Start.

6. After the pause, the protocol will continue to the end.

7. After the run is completed, remove the plates and store the purified total RNA.

【Basic Information】

	Guangzhou Magen Biotechnology Co., Ltd. Room 401, Building D, No. 7, Jingye 3rd Street, Yushu Industrial Park, Guangzhou Hi-Tech Industrial Development Zone, Huangpu District, Guangzhou, 510663, China www.magen-tec.com 86-20-3855 5004
EC REP	**StateLab GmbH** Friedrich-Ebert-Strasse 7, 58642 Iserlohn, Germany