【Product Name】 MagPure Circulating DNA Maxi Kit

【Product specifications】10Preps/Kit， 50 Preps/Kit

【Intended Use】

This Kit is designed for purification of high quality circulating DNA (cfDNA) from 1-8ml cell-free body fluids (such as plasma, serum). The purified DNA is suitable for direct use in downstream applications such as PCR, real-time PCR, Biochip analysis and NGS.

【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. 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.	IVD5435-10	IVD5435	Composition
MagPure Particles F	3.0 ml	14 ml	Magnetic Particles
Carrier RNA	310 ug	310 ug	Poly A
Buffer SDS	3 ml	15 ml	SDS
Proteinase K	60 mg	240 mg	Protease
Protease Dissolve Buffer	5 ml	15 ml	Glycorel/Tris/$CaCl_2$
Buffer MLK	120 ml	500 ml	Guanidine Salt
Buffer MAW1	50 ml	250 ml	Guanidine Salt
Buffer MW2*	10 ml	50 ml	Tris/NaCl
Elution Buffer	5 ml	60 ml	Tris

【Storage conditions and Validity】

Proteinase K, Carrier RNA and MagPure Particles F 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 dry at room temperature (15–25°C) and are stable for at least 18 months under these conditions.The entire kit can be stored at 2–8°C, but in this case buffers should be redissolved before use. Make sure that all buffers are at room temperature when used.

【Preparation before Use】

- Add 3.0ml (10Preps) or 12ml (50Preps) Protease Dissolve Buffer to the Proteinase K, and store at -20~8℃ after dissolve.
- Add 0.31ml ml Elution Buffer to the Carrier RNA, and store at -20℃ after dissolve. Optional: Add 0.1~1μg each prep. Carrier RNA can reduce the adsorption of consumables on DNA, but carrier RNA can affect the quantification of qubit.
- Dilute Buffer MW2 with 40 ml (10 Preps) or 200ml (50 Preps) 100% ethanol.

【Manual Protocol for 1-4ml】

1. The Volume of Sample, Proteinase K, MagPure Particles F, Buffer MLK.

Sample Volume	1ml	2ml	3ml	4ml
Proteinase K	50 μl	100 μl	150 μl	200 μl
MagPure Paritlce F	60 μl	120 μl	180 μl	240 μl
Buffer SDS	50 μl	100 μl	150 μl	200 μl
Buffer MLK	2.0 ml	3.8 ml	5.8 ml	7.8 ml
Elution Buffer	35-50 μl	50~60 μl	70~100 μl	70~100 μl
Carrier RNA	0.1~0.5μg			

2. **Transfer 50~200μl Proteinase K and 1~4ml Plasma or serum to a new 15 ml centrifuge tube.**
3. **Add 50~200 μl SDS to the sample and mix well. Incubate at 55-60°C for 30-60 min.**
4. **Add 2.0~7.8ml Buffer MLK and 60-240μl MagPure Particles F to the sample.** Mix upside down for 10~15 minutes at room temperature. Place the tube to the magnetic stand for 3~5 minutes until the beads have formed a tight pellet. Then remove the supernatant.
5. **Add 2000μl Buffer MAW1** 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.
6. **Add 2000μl Buffer MAW1** 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.
7. **Add 3000μl Buffer MW2,** 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.
8. **Add 1000μl Buffer MW2,** and vortex for 15 seconds to re-suspend beads. Transfer the mxiture to 1.5ml microcentrifuge tube and place the tube to the magnetic stand for 1 minute until the beads have

form a tight pellet. Then remove the supernatant.

9. Centrifuge shortly to collect liquid on the tube. Place the tube to the magnetic stand and remove all the liquid carefully.
10. Dry at 37-55°C for 5~10 minutes.
11. Add 50~100μl Elution Buffer/Low TE and re-suspend the beads by vortex. Sit at room temperature for 10 minutes. Shake 3-5 times to dissolve DNA from magnetic particles more efficiently.
12. Place the tube to the magnetic stand for 1 minutes. Transfer the supernatant containing the purified DNA to a clean 1.5ml centrifuge tube.

【Manual Protocol for 5-8ml】

1. The Volume of Sample, Proteinase K, MagPure Particles F, Buffer MLK.

Sample Volume	5ml	6ml	7ml	8ml
Proteinase K	250 μl	300 μl	300 μl	300 μl
Buffer SDS	250 μl	300 μl	350 μl	400 μl
Buffer MLK	9 ml	11ml	13 ml	14 ml
MagPure Particles F	240 μl	240 μl	240 μl	240 μl
Elution Buffer	70~100 μl	70~100 μl	70~100 μl	70~100 μl
Carrier RNA	0.1~0.5μg			

2. **Transfer 250~300 μl Proteinase K and add 5~8ml Plasma or serum to a new 50 ml centrifuge tube.**
3. **Add 250~400 μl SDS to the sample and mix well. Incubate at 55-60°C for 30-60 min.**
4. Add 9.0-14.0ml Buffer MLK to the sample, Mix well by inverting for 15 times.
5. **Transfer one half of lysate into a new 15 ml centrifuge tube and dd 200μl MagPure Partilces F to the sample.** Mix upside down for 6 minutes at room temperature. Place the tube to the magnetic stand for 3 minutes until the beads have formed a tight pellet. Then remove the supernatant.
6. Transfer the remaining of lysate into the centrifuge tube containing the beads. Vortex to resupsend beads and mix upside down for 6 minutes at room temperature. Place the tube to the magnetic stand for 3 minutes until the beads have formed a tight pellet. Then remove the supernatant.
7. Follow step 5-12 of Manual protocol for 1-4 ml.

【Auto Purify by KingFisher Flex for 1~6ml Sample】

1. The Volume of Sample, Proteinase K, MagPure Particles F, Buffer MLK.

Sample Volume	1 ml	2 ml	3 ml	4 ml	5 ml	6 ml
Proteinase K	50 μl	100 μl	150 μl	200 μl	250 μl	300 μl
Buffer SDS	50 μl	100 μl	150 μl	200 μl	250 μl	300 μl
MagPure Paritlce F	60 μl	90 μl	150 μl			
Buffer MLK	1.9 ml	3.5 ml	5.2ml	6.8 ml	8.8 ml	10.0 ml
Elution Buffer	50 ~60μl		75 ~90μl			
Carrier RNA	0.1~0.5μg					

2. Transfer 50~300 μl Proteinase K, 50~300 μl Buffer SDS and 1~6ml Plasma or serum to 15~50ml centrifuge tube. Mix well and incubate at 55-60°C for 30-60 min.
3. Transfer the lysate itno 24 well plate.

Volume	Sample Plate		
	Sample A	Sample B	Sample C
1 ml	~3.0ml Lysate	Null	Null
2 ml	2.8ml Lysate	2.8ml Lysate	Null
3 ml	4.3ml Lysate	4.3ml Lysate	Null
4 ml	3.8ml Lysate	3.8ml Lysate	3.8ml Lysate
5 ml	4.7 ml Lysate	4.7 ml Lysate	4.7 ml Lysate
6 ml	5.3 ml Lysate	5.3 ml Lysate	5.3ml Lysate

4. Transfer the wash buffer into 24 well plate.

Wash Plate 1	2000μl BufferMAW1、60~150μl MagPure Particle F、24-Tip
Wash Plate 2	2000μl Buffer MAW1
Wash Plate 3	4500μl Buffer MW2
Wash Plate 4	500μl Absolute ethanol
Elution plate	75~90μl Elution Buffer

5. Turn on the machine, start the corresponding program.
6. Place the 24-well plate into the instrument as prompted.
7. Finish the operation after 60~90 minutes.
8. Remove the 24-well plate and magnetic jacket.
9. Store the Elute product at -20~8℃.