【Product Name】 MagPure Circulating DNA Maxi Kit

【Product specifications】 10 Preps/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	110 ug	110 ug	Poly A
Buffer SDS	3 ml	15 ml	SDS
Proteinase K	50 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 24 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 2.5ml (10Preps) or 12ml (50Preps) Protease Dissolve Buffer to the Proteinase K, and store at -20~8℃ after dissolve.
- Add 1.1ml Elution Buffer to the Carrier RNA, and store at -20℃ after dissolve.
- Dilute Buffer MW2 with 40 ml (10 Preps) or 200ml (50 Preps) 100% ethanol.

【Manual Protocol for 1-4ml】

1. The Volume of Sample and Proteinase K/MagPure Particles F/Buffer SDS/ Buffer MLK. Use.

Sample Volume	1ml	2ml	3ml	4ml
Proteinase K	50 µl	100 µl	150 µl	200 µl
MagPure Paritlce F	75 µl	150 µl	200 µl	300 µl
Buffer SDS	50 µl	100 µl	150 µl	200 µl
Buffer MLK	2.0 ml	3.8 ml	5.6 ml	7.5 ml
Elution Buffer	35-50 µl	50~60 µl	70~100 µl	70~100 µl
Carrier RNA (optinal)	1~5µl (0.1~0.5µg)			

2. **Transfer suitable volume of Proteinase K and Carrier RNA into a new 15 ml centrifuge tube** (refer to above table).**Then add 1~4ml serum or plasma to the tube.**

   Note: Carrier RNA may affect quibit reading. But Carrier RNA can reduce the adsorption of consumables and stabilize cfDNA yield. We recommend to use 0.2~0.5µg Carrier RNA per reaction.

3. **Add Buffer SDS to the sample and mix well.** Incubate at 55-60°C for ~30 min and mix upside down several times. Stay at room temperature for ~5 min.

4. **Add Buffer MLK and MagPure Particles F to the sample.** Mix upside down for 10 min 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. **Repeat Step 5 once**.

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. 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℃ for 5~10 minutes.
11. Add 35~100μl Elution Buffer/Low TE to the sample and re-suspend the beads by vortex. Stay 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, double binding】

1. The Volume of Sample and Proteinase K/MagPure Particles F/Buffer SDS/ Buffer MLK use.

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	300 μl	350 μl	400 μl
Elution Buffer	70~100 μl	70~100 μl	70~100 μl	70~100 μl
Carrier RNA (optinal)	1~5μl (0.1~0.5μg)			

2. **Transfer suitable volume of Proteinase K and Carrier RNA into a new 50 ml centrifuge tube.** (refer to above table). **Then add 5~8ml Plasma or serum to the tube.**

   Note: Carrier RNA may affect quibit reading. But Carrier RNA can reduce the adsorption of consumables and stabilize cfDNA yield. We recommend to use 0.2~0.5μg Carrier RNA per reaction
3. **Add Buffer SDS to the sample and mix well.** Incubate at 55-60℃ for 30~60 min and mix upside down for several times. Stay at room temperature for 5~10 min.
4. **Add Buffer MLK to the sample.** Mix well by inverting for 15 times.
5. **Transfer half volume of lysate into a new 15 ml centrifuge tube and add 240~400μl 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 re-supsend 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	75 μl	100 μl	150 μl			200 μl
Buffer MLK	1.9 ml	3.5 ml	5.2ml	7.0 ml	8.8 ml	10.0 ml
Elution Buffer	50 ~60μl		75 ~90μl			
Carrier RNA	0.1~0.5μg					

2. Transfer suitable volume of Proteinase K, Buffer SDS and Plasma or serum into a new15~50ml centrifuge tube (referring to above table). Mix well and incubate at 55-60℃ for 30-60 min.
3. Add Buffer MLK to the sample, Mix well by inverting for 5~10 times.
4. 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

5. 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

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