

# MolPure™ Magnetic Cell-Free DNA Kit

## Product Information

Product Name	Cat#	Specification
MolPure™ Magnetic Cell-Free DNA Kit	18381ES50	50 T

## Product Description

MolPure™ Magnetic Cell-Free DNA Kit is suitable for the extraction of cell-free DNA from 1-5 mL of plasma and serum samples. This product uses unique magnetic beads and a carefully optimized buffer system to maximize the purification and recovery of free DNA. The extracted nucleic acid has high yield, stable and reliable quality, and maximizes the removal of other inhibitors such as protein, which is suitable for various downstream application experiments, such as fluorescence quantitative PCR and next-generation sequencing.

## Product Components

Category	Component number		18381ES50 (50 servings/box)
Part I	18381-A	Proteinase K solution (20mg/mL)	1 mL/piece×2 piece
	18381-B	Magnetic Bead Suspension	800 µL/piece×1 piece
	18381-C	Lysate	8 mL/bottle×1 bottle
Part II	18381-D	Binding solution	80 mL/bottle×1 bottle
	18381-E	Washing liquid A*	22 mL/bottle×1 bottle (add 33 mL ethanol)
	18381-F	Washing liquid B*	22 mL/bottle×1 bottle (add 88 mL ethanol)
	18381-G	Eluent	5 mL/bottle×1 bottle

## Shipping and Storage

Part I components are transported at room temperature, stored at 4°C, and valid for 12 months.

Part II components are transported at room temperature, stored at room temperature, and valid for 12 months.

## Cautions

1. Pay attention to observe whether each solution has precipitation or turbidity (especially when the room temperature is low temperature environment such as winter), you can heat the solution at 37°C until the solution is clear to avoid affecting the use effect.
2. Avoid placing the magnetic beads below 4°C and avoid repeated freezing and thawing, otherwise the yield of DNA will decrease.
3. There may be residual magnetic beads during elution, so try to avoid aspirating magnetic beads when drawing samples.
4. For your safety and health, please wear a lab coat and disposable gloves.
5. This product is for scientific research purposes only.

## Preparation

1. Self-provided equipment and reagents: magnetic separation rack (for 2 mL and 15 mL centrifuge tubes), water bath or metal bath, vortex shaker, rotary mixer, 2 mL and 15 mL centrifuge tubes, absolute ethanol, etc.
2. Before the first use, add the volume of absolute ethanol indicated on the label to the bottles of washing solution A\* and washing solution B\*, mix thoroughly before use, and make a mark. Cap the bottle tightly after each use to maintain the ethanol level in the bottle.

## Manual extraction method

**Before use, please add absolute ethanol to Washing Solution A and Washing Solution B. Please add volume according to the label on the bottle.**

1. Sample processing. Add the following components to a 15 mL centrifuge tube according to the volume of the sample.

reagent	Plasma/serum volume		
	1 mL	2 mL	5 mL
Proteinase K solution	40 $\mu$ L	80 $\mu$ L	200 $\mu$ L
Lysate	150 $\mu$ L	300 $\mu$ L	750 $\mu$ L
Plasma/serum	1 mL	2 mL	5 mL

2. After briefly vortexing and mixing, heat in a 63°C water bath for 20 minutes, during which time it needs to be inverted and mixed.

3. According to the volume of the sample, add the binding solution and magnetic bead suspension to the 15 mL centrifuge tube according to the following table (**the magnetic beads should be fully vortexed before adding the magnetic beads**).

reagent	Plasma/serum volume		
	1 mL	2 mL	5 mL
Binding solution	1500 $\mu$ L	3000 $\mu$ L	7500 $\mu$ L
Magnetic Bead Suspension	15 $\mu$ L	30 $\mu$ L	75 $\mu$ L

4. After vortexing briefly, place the centrifuge tube on a rotary mixer and shake for 10 minutes (or stand for 10 minutes, and vortex for 10 seconds every 2 minutes).

5. Place the 15 mL centrifuge tube on the magnetic stand for 5 minutes until the solution becomes clear and the magnetic beads are completely adsorbed on the tube wall. Carefully remove the supernatant.

6. Remove the 15 mL centrifuge tube from the magnetic stand, add 1 mL of washing solution A\* (**check whether ethanol is added before use**), vortex and mix, and transfer all the liquid and magnetic beads in the 15 mL centrifuge tube to a new 1.5 mL, keep the 15 mL centrifuge tube.

7. Place the 1.5 mL centrifuge tube on the magnetic stand for 2 minutes until the solution becomes clear and the magnetic beads are completely adsorbed on the tube wall, and the supernatant is sucked back into the 15 mL centrifuge tube from the previous step.

8. Vortex and mix the 15 mL centrifuge tube, transfer all the liquid after rinsing the tube wall to the 1.5 mL centrifuge tube in the previous step, and magnetically absorb it again on the magnetic stand for 2 min until the solution becomes clear and the magnetic beads are completely adsorb on the tube wall and discard the supernatant.

9. Add 1 mL of Washing Solution B\* to a 1.5 mL centrifuge tube (**check whether ethanol is added before use**), vortex and mix for 20 s, centrifuge briefly and place on a magnetic stand for 2 min until the solution becomes clear and the magnetic beads are completely adsorb on the tube wall and discard the supernatant.

10. Repeat step 9 again.

11. After a brief centrifugation, place the centrifuge tube on the magnetic stand again. After the magnetic beads are adsorbed on the tube wall, use a pipette to suck out the liquid remaining at the bottom of the tube.

12. Open the cap of the tube, dry at room temperature for 5-10 min or heat to dry at 60°C until the ethanol evaporates (dry until the surface of the magnetic beads just cracks, excessive drying will affect the nucleic acid elution effect).

13. Take the above air-dried 1.5 mL centrifuge tube and add the eluent to the centrifuge tube according to the table below.

reagent	Plasma/serum volume		
	1mL	2mL	5mL
Eluent	30-50 $\mu$ L	50-100 $\mu$ L	150-200 $\mu$ L

14. Vortex to mix, after a brief centrifugation, incubate at 65°C for 5 min. During this period, shake and mix to improve the elution effect.

15. After a brief centrifugation, place the centrifuge tube on a magnetic stand and let the magnetic beads completely absorb.

Carefully transfer the DNA solution to a new centrifuge tube, taking care not to absorb the magnetic beads.

16. Nucleic acid solution should be stored at -20°C, and long-term storage should be stored at -80°C.

### Semi-automatic extraction method

For use with automated instruments, take Aosheng Auto-Pure32A Automatic Nucleic Acid Extractor as an example.

1. Add samples and reagents to the corresponding positions according to the table below

Notice:

(1). Confirm that washing solution A\* and washing solution B\* have been added with the corresponding volume of absolute ethanol;

(2). Reagents should be added to the wells in the 1/7th column in strict accordance with the order of the table.

(3). The magnetic bead suspension should be fully resuspended on a vortex mixer before use. After adding samples 4-5 times at a time, it is recommended to mix again before adding samples.

Location	Reagent name	Volume/location
1/7 column	Proteinase K solution	20 μL
	Plasma/serum	350 μL
	Lysate	40 μL
	Binding solution	500 μL
2/8 column	Washing liquid A*	700 μL
3/9 column	Washing liquid B*	700 μL
	Magnetic Bead Suspension	20 μL
4/10 column	Washing liquid B*	700 μL
5/11 column	-	-
6/12 column	Eluent	70 μL

2. According to the position of the extractor, place the above 96-well pre-installed plate correctly, and place the 8-pole magnet sleeve.

3. Run the following program. After the program is over, transfer the eluate to a new centrifuge tube. The solution can be stored at -20°C for short-term storage and -80°C for long-term storage.

The extraction procedure of the extraction instrument of Aosheng Auto-Pure32A

Step	Locate	Mix (min)	Adsorpte (sec)	Waite(min)	Volum e(μL)	Mixing speed(1-10)	Tempe rature( °C)	Mixed position(0-10 0%)	Mixed amplitude(1-1 00%)	Magnetic position(0-10 0%)	Magnetic speed(1-10)
transfer beads	3	0.3	60	0	700	5	/	0	80	0	1
combine	1	15	80	0	910	3	70	0	80	0	1
cleaning 1	2	2	30	0	700	7	/	0	80	0	1
cleaning 2	3	2	30	0	700	5	/	0	80	0	1
cleaning 3	4	1	30	2.5	700	5	/	0	80	0	1
elution	6	6	0	0	70	4	60	0	80	0	1
elution	6	2	80	0	70	7	60	0	80	0	1
Discard beads	3	0.2	0	0	700	5	/	0	80	0	1

If it is to be used with other mainstream automated instruments, the program can be obtained from the technical support

