

MolPure™ Magnetic FFPE DNA Kit

Product Information

Product Name	Cat#	Specification
MolPure™ Magnetic FFPE DNA Kit	18371ES50	50 T

Product Description

MolPure™ Magnetic FFPE DNA Kit is suitable for DNA extraction from formalin-fixed, paraffin-embedded tissues. Using unique magnetic beads and carefully optimized buffer system, it can maximize the separation and purification of high-purity DNA. The dewaxing solution used in this kit is safer, simpler and faster than xylene; the extracted genome has good integrity, high purity, stable and reliable quality, and is suitable for various downstream application experiments, such as PCR and next-generation sequencing. This product can be used in conjunction with the magnetic bead method automated extraction instrument and the pipetting automated instrument to achieve high-throughput extraction of nucleic acids.

Product Components

Category	Component number	Component name	18371ES50 (50 servings/box)
Part I	18371-H	Proteinase K solution (20mg/mL)	1 mL/piece×1 piece
	18371-A	Magnetic Bead Suspension	1 mL/piece×1 piece
	18371-B	Dewaxing solution	20 mL/bottle×1 bottle
	18371-C	Lysate	10 mL/bottle×1 bottle
Part II	18371-D	Binding solution	20 mL/bottle×1 bottle
	18371-E	Washing liquid A	16 mL/bottle×1 bottle (add 24mL ethanol)
	18371-F	Washing liquid B	16 mL/bottle×1 bottle (add 64mL ethanol)
	18371-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 take a water bath at 37°C until the solution is clear to avoid affecting the use effect.
2. The magnetic beads should not be placed in an environment below 4°C, and repeated freezing and thawing will be avoided, otherwise the yield of DNA will be reduced.
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, 32 or 96-channel automated nucleic acid extraction instrument, water bath or metal bath, vortex shaker, 1.5 mL centrifuge tube, 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 (18371-E) and

Washing Solution B (18371-F), 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

a Paraffin section: Take 5-8 paraffin sections (5-10 μm thick, $1 \times 1 \text{ cm}^2$ size).

b Paraffin-embedded samples: use a scalpel to scrape a tissue sample of approximately 10 mg (try to remove excess paraffin).

Note: If the sample surface is exposed to air, discard the 2-3 pieces that are exposed to the air.

c Samples in fixatives such as formalin: take about 10 mg of sample, cut it into small pieces with a scalpel, put it in a 1.5 mL centrifuge tube, and add 500 μL of PBS solution (pH7.4)

Vortex, centrifuge at 12,000 rpm ($\sim 13,400 \times g$) for 1 min at room temperature, discard the supernatant, and repeat 3 times.

2. Place the sample in a 1.5 mL centrifuge tube, add 400 μL of deparaffinization buffer, 200 μL of lysis buffer, and 20 μL of proteinase K, and mix by vortexing for 10 seconds.

3. Incubate at 65°C for 1 hour until the sample is completely lysed. In this step, the sample may not be completely lysed, and you can go directly to the next step.

4. Incubate at 90°C for 1 hour.

Note: This step should be carried out in strict accordance with the heating temperature and time given in the instructions.

5. After centrifugation at 12,000 rpm for 1 min, use a 200 μL pipette tip to carefully pipette the lower 200 μL incubation product along the tube wall and transfer it to a new 1.5 mL centrifuge tube.

6. (Optional) If the residual RNA affects the subsequent experiments, add 5 μL of RNase A solution (100 mg/mL) (self-provided, Yeasen Cat No.10406), shake and mix, and leave at room temperature for 5-10 min.

7. Add 400 μL of binding solution and vortex to mix.

8. Add 20 μL of magnetic bead suspension, vortex for 20 seconds, incubate on a rotary mixer for 10 minutes, or let stand for 10-15 minutes, and vortex for 20 seconds every 2-3 minutes.

Note: The magnetic bead suspension needs to be fully vortexed before use to ensure that the magnetic beads are completely resuspended.

9. After a brief centrifugation, place the centrifuge tube on a magnetic rack and let it stand until the magnetic beads are completely adsorbed, then discard the supernatant.

10. Add 700 μL of Washing Solution A (please check whether absolute ethanol has been added before use), shake and mix for 1 min to ensure that the magnetic beads are dispersed.

11. After a brief centrifugation, place the centrifuge tube on a magnetic stand and let the magnetic beads completely adsorb, then carefully aspirate the liquid.

12. Add 700 μL of Washing Solution B (please check whether absolute ethanol has been added before use), shake and mix for 1 min to ensure that the magnetic beads are dispersed.

13. After a brief centrifugation, place the centrifuge tube on a magnetic rack and let it stand until the magnetic beads are completely adsorbed. Carefully aspirate the liquid.

14. Repeat steps 12 and 13.

15. Aspirate the remaining liquid as much as possible, and place the centrifuge tube on a magnetic stand to dry for 5-10 minutes, or heat and dry at 60°C until the ethanol evaporates.

Note: Air dry until the surface of the magnetic beads is just cracked. Excessive drying is not conducive to nucleic acid elution.

16. Remove the centrifuge tube from the magnetic stand, add 50-100 μL of eluent, and mix by shaking. If the magnetic beads are clumped, you can use a pipette to blow. After a brief centrifugation, incubate at 60°C for 5 minutes, and shake and mix well before the period to improve the elution effect.

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

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

18. 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. Sample processing

- a. Paraffin section: Take 5-8 paraffin sections (5-10 μm thick, $1 \times 1 \text{ cm}^2$ size).
- b. Paraffin-embedded samples: use a scalpel to scrape about 10 mg of tissue samples (try to remove excess paraffin).

Note: If the sample surface is exposed to air, discard the 2-3 pieces that are exposed to the air.

c. Samples in fixatives such as formalin: take about 10 mg of sample, cut it into small pieces with a scalpel, put it in a 1.5 mL centrifuge tube, add 500 μL PBS (pH7.4)Vortex, centrifuge at 12,000 rpm ($\sim 13,400 \times g$) for 1 min at room temperature, discard the supernatant, and repeat 3 times.

2. Put the sample in a 1.5 mL centrifuge tube, add 400 μL of deparaffinization buffer, 200 μL of lysis buffer, and 20 μL of proteinase K, and mix by vortexing for 10 seconds.

3. Incubate at 65°C for 1 hour until the sample is completely lysed.

4. Incubate at 90°C for 1 hour.

Note: This step should be carried out in strict accordance with the heating temperature and time given in the instructions.

5. After a brief centrifugation, remove the bottom 200 μL of the incubation product and transfer it to a new 1.5 mL centrifuge tube.

6. (Optional) If the residual RNA affects subsequent experiments, add 5 μL of RNase A (100 mg/mL) (self-provided, Yeasen Cat No. 10406) solution, shake and mix, and leave at room temperature for 5-10 min.

7. Add the appropriate reagents to the 96-well deep-well plate according to the following table:

Location	Reagent name	Volume/location
1/7 column	Samples	200 μL
	binding solution	400 μL
2/8 column	Washing solution A	700 μL
3/9 column	Washing solution B	700 μL
	Magnetic Bead Suspension	20 μL
4/10 column	Washing solution B	700 μL
5/11 column	—	—
6/12 column	Eluent	70 μL

Note: The magnetic bead suspension needs to be fully resuspended or fully inverted on a vortex mixer before use. After adding samples 4-5 times at one time, it is recommended to mix again before adding samples.

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

9. 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)	Volume(μL)	Mixing speed(1-10)	Temperatu re(°C)	Mixed position(0-10 0%)	Mixed amplitude(1-100%)	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	600	3	/	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	65	0	80	0	1
elution	6	2	80	0	70	7	65	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 department of Yeasen Biotechnology.