VAMNE MagUltra FFPE DNA Extraction Kit

DM601

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Instruction for Use

Version 24.1

Contents

01/Product Description

This kit employs a safe, non-toxic, and environmentally friendly deparaffinization solution along with an efficient lysis/de-crosslinking reagent, which can lyse and release trace DNA in paraffin embedded sections and tissues. The high-affinity magnetic beads used in the kit can adsorb nucleic acids in a high-salt buffer and release nucleic acids in a low-salt elution buffer, thereby achieving rapid separation and purification of nucleic acids. The obtained products has good integrity and amplifiability, suitable for downstream applications such as PCR, next-generation sequencing, hybridization capture, etc. **This kit can be used with automated nucleic acid extraction instrument for high-throughput extraction.**

02/Components

	Components	DM601-01 (50 rxns)	DM601-02 (100 rxns)
BOX 1	Proteinase K	2 ml	4 ml
	MagUltra Beads C	1.5 ml	2 × 1.5 ml
BOX 2	Deparaffinization Solution	40 ml	80 ml
	Buffer L/D	10 ml	20 ml
	Buffer FB	12 ml	24 ml
	Buffer WA	24 ml	48 ml
	Elution Buffer	8 ml	16 ml

03/Storage

BOX 1: Store at 2 ~ 8°C and ship on ice pack.

BOX 2: Store at 15 ~ 25°C and ship at room temperature.

04/Applications

0.5 - 5 paraffin sections (10 μm thick with approximately 30 mm^2 tissue area); <10 mg formalin-fixed and paraffin-embedded tissues.

05/Self-prepared Materials

Isopropanol, absolute ethanol, PBS, RNase A (100 mg/ml) (Vazyme #DE111), 1.5 ml or 2 ml Nuclease-free centrifuge tube;

magnetic rack, vortex mixer, centrifuge, water bath.

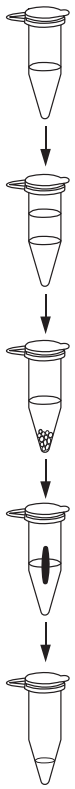
06/Notes

1. Before the first use of the reagent, **add isopropanol with labeled volume to the Buffer FB bottle (14 ml isopropanol for DM601-01, 28 ml isopropanol for DM601-02), and add absolute ethanol with labeled volume to the Buffer WA bottle (32 ml absolute ethanol for DM601-01, 64 ml absolute ethanol for DM601-02).** Mix thoroughly and make proper labeling.

2. Before use, check each component for precipitation. If precipitation is present, incubate at 37°C in a water bath for 30 min to re-dissolve, and mix thoroughly before use.

3. Ordinary 1.5 ml centrifuge tube may fall off when heated at 90°C. And it can be fixed with explosion-proof clamps.

07/Mechanism & Workflow



Sample pre-processing

◇ Paraffin sections or embedded tissue: Take samples, add 800 µl Deparaffinization Solution to the sample, and vortex vigorously. Briefly centrifuge, then incubate at 56°C for 5 min, and vortex vigorously for 15 sec.

◇ Formalin-fixed tissue:Take approximately 10 mg of the sample. Add 500 µl PBS solution and vortex, centrifuge at 12,000 rpm (13,500 × g) for 1 min at room temperature. Discard the supernatant (repeat three times).

Lyse, de-crosslinking and nucleic acid binding:

a. Add 40 µl Proteinase K and 200 µl Buffer L/D to the sample, vortex for 5 - 10 sec, then briefly centrifuge.
b. Incubate at 56°C for 60 - 180 min (until samples is completely digested)
c. Incubate at 90°C for 60 min (to remove DNA and protein crosslinking).
d. Transfer the lower digestion solution to a new centrifuge tube. Add 400 µl of Buffer FB and 30 µl of MagUltra Beads C to the digestion solution, vortex to mix for 15 seconds, and let it stand at room temperature for 3 minutes, inverting the tube several times during this period.

Washing and elution:

a. Remove protein: Wash twice with 500 µl Buffer WA.
b. Remove residual ion: Wash twice with 500 µl 80% ethanol.
c. Remove ethanol: Discard supernatant, air dry for 5 - 10 min.
d. Add 50 - 100 µl Elution Buffer, vortex to resuspend beads and incubate at 56°C for 5 min to elute nucleic acid.

Fig 1. Workflow of VAMNE MagUltra FFPE DNA Extraction Kit

08/Experiment Process

08-1/Sample pre-treatment

◇ **Paraffin section**

1. Take 0.5 - 5 pieces of paraffin sections (10 µm thick with approximately 30 mm^2 tissue area), scrape the sliced tissue off with a clean blade and transfer it to a 1.5 ml centrifuge tube.

▲ Removing excess paraffin or cutting the sample into smaller pieces using scissors or a blade facilitates subsequent deparaffinization.

2. Add **800 µl Deparaffinization Solution** to the sample, vortex vigorously for 5 sec. Briefly centrifuge, then incubate at 56°C for 5 min, vortex vigorously for 15 sec, and proceed to **08-2/Lyse, de-crosslinking and nucleic acid binding**.

◇ **Paraffin embedded tissue**

1. Scrape approximately 10 mg of sample tissue with a surgical knife and transfer it to a 1.5 ml centrifuge tube.

▲ Removing excess paraffin or cutting the sample into smaller pieces using scissors or a blade facilitates subsequent deparaffinization.

2. Add **800 µl of Deparaffinization Solution** to the sample, vortex vigorously for 5 sec. Briefly centrifuge. Incubate at 56°C for 5 min. Vortex vigorously for 15 sec, and proceed to **08-2/Lyse, de-crosslinking and nucleic acid binding**.

◇ **Formalin-fixed tissue**

1. Take approximately 10 mg of the sample, cut it into small pieces with a surgical knife, and place it in a 1.5 ml centrifuge tube.

2. Add **500 µl PBS** solution and vortex, centrifuge at 12,000 rpm (13,500 × g) for 1 min at room temperature. Discard the supernatant.

3. Repeat step 2 three times, and proceed **08-2/Lyse, de-crosslinking and nucleic acid binding**.

08-2/Lyse, de-crosslinking and nucleic acid binding

1. Add **40 µl Proteinase K and 200 µl Buffer L/D** to the sample, vortex for 5 - 10 sec, then briefly centrifuge.

2. Place the centrifuge tube in a water bath or metal bath at 56°C for 60 - 180 min (until samples is completely digested), then incubate at 90°C for 60 min.

3. Briefly centrifuge the tube to collect any droplets on the tube walls. Transfer the lower layer of digestion solution (approximately 220 µl) to a new centrifuge tube.

▲ When transferring the lower layer of digestion solution, please avoid absorbing the upper layer deparaffinization solution. After transferring the digestion solution, let it stand to allow the digestion solution to return to room temperature.

4. (Optional) If you want to remove RNA from the sample, after the sample has returned to room temperature, add **2 µl of RNase A (100 mg/ml)**, vortex gently and let it stand at room temperature for 3 - 5 min.

5. Add **400 µl Buffer FB and 30 µl MagUltra Beads C** to the digestion solution, vortex for 15 sec, let it stand at room temperature for 3 min, and mix by inversion several times during the incubation.

6. Briefly centrifuge, then place the centrifuge tube on a magnetic rack for 2 min to allow complete adsorption of magnetic beads. Carefully discard the supernatant.

08-3/Washing and elution

1. Add **500 µl Buffer WA**, vortex for 15 sec. Briefly centrifuge and transfer to a magnetic rack for 2 min to allow the magnetic beads to fully be adsorbed. And carefully discard the supernatant.
2. Repeat step 1 once.
3. Add **500 µl 80% ethanol**, vortex for 15 sec.Briefly centrifuge, then transfer to a magnetic rack and adsorb for 2 min, carefully discard the supernatant.
4. Repeat step 3 once.
5. Briefly centrifuge and transfer to a magnetic rack for 1 min, carefully discard all supernatant.
6. Open the tube cap, air dry for 5 - 10 min.
7. Add **50 - 100 µl Elution Buffer**, vortex the magnetic beads, and incubate at 55°C for 5 min. Mix by inversion several times every 2 min during this period. Briefly centrifuge, then transfer the centrifuge tube to the magnetic rack for 2 min to adsorb. Transfer the supernatant to a new centrifuge tube, and store the obtained product at -20°C.

09/FAQ & Troubleshooting

FAQ	Reasons	Solutions
Low DNA yields	1. Improper sample input	It is recommended to extract the sample according to the recommended input amount.
	2. Improper storage of Proteinase K resulting in reduced activity or inactivation	To confirm the storage conditions of Proteinase K or use new Proteinase K for the digestion reaction, it is recommended to use Proteinase K (20 mg/ml) (Vazyme #DE102).
	3. Sample is not fully digested	Extend the lysis time in 56°C water bath appropriately. Increase inversion mixing times to be fully digest samples.
	4. Over-drying of beads	If the magnetic bead cracks, it indicates that the magnetic bead is over-drying.
	5. Elution buffer issues	Please elute with Elution Buffer. If ddH_2O or another elution buffer is used, make sure that its pH is between 7.5 - 8.5.
	6. Incomplete elution	The beads are not thoroughly mixed after the addition of Elution Buffer. Extend the shaking time as appropriate until the beads are thoroughly mixed.
Low DNA purity	1. Protein contamination: Buffer WA washing is insufficient or washing times are insufficient.	Wash three times or purify with VAHTS DNA Clean Beads (Vazyme #N411).
	2. Salt ion contamination: 80% ethonal washing is insufficient or washing times are insufficient.	
	3. Residual ethanol	When drying, tap the table with the magnetic rack to collect the residual liquid to the bottom, and pipette and discard it with the 20 µl tips; Incubate at 50°C water bath or metal bath for 5 - 10 min to promote the volatilization of residual ethanol.
RNA contamination	1. RNA is extracted	After decrosslinking at 90°C, when the sample returned to room temperature, add 2 µl of RNase A (100 mg/ml) to the sample, and incubate at room temperature for 3 - 5 min.
The magnetic beads are not firmly attached	1. The magnetic capacity of the magnetic rack is insufficient	Replace the magnetic rack with a higher magnetic performance.

Appendix I: The use of automated instruments, using Vazyme #VNP-32P as an example.

1. Reagent preparation, add the corresponding reagents to the 96-well deep well plate according to the following table.

Well	1/7 Column	2/8 Column	3/9 Column	4/10 Column	5/11 Column	6/12 Column
Reagent	Buffer FB	Buffer WA	80% Ethanol	80% Ethanol	ddH_2O and magnetic beads	Elution Buffer
Volume (µl)	400	500	500	500	270 and 30	50 - 100

2. The processes of deparaffinization, lysis, and de-crosslinking for the samples are consistent with the manual extraction method.

3. Add approximately 220 µl of the lower layer digestion solution to columns 1 and 7 of the 96-well plate, ensuring to avoid cross-contamination.

4. Place the 96-well plate into the VNP-32P automated nucleic acid extraction instrument. Attach the magnetic rod sleeves, ensuring that they are properly installed and securely in place.

5. Run the following program:

Step	Plate Position	Name	Mixing Time (min)	Adsorption Time (min)	Waiting Time (min)	Volume (µl)	Mixing Speed	Temperature (°C)	Mixing Position	Mixing Amplitude	Adsorption Position	Adsorption Speed
1	5	Move-beads	0.5	0.5	0	300	10	OFF	10	80	0	5
2	1	Bind	10	1	0	650	10	OFF	10	100	0	5
3	2	Wash 1	1	0.5	0	500	10	OFF	10	100	0	10
4	3	Wash 2	1	0.5	0	500	6	OFF	10	100	0	10
5	4	Wash 3	1	0.5	1	500	6	OFF	10	100	0	10
6	6	Elution	10	1	0	100	10	65	10	100	0	10
7	5	Discard beads	0.1	0	0	300	5	OFF	0	80	0	1
Other settings (in the Option menu): Heating settings (heating and action start at the same time) Adsorption settings (three-stage adsorption)												

6. After the automated program is completed, transfer the elution solution from columns 6 and 12 (paying attention to the effective working wells) to clean, nuclease-free centrifuge tubes. If not used immediately, store the eluted product at -20°C.