VAMNE MagUltra Blood Genomic DNA Extraction Kit

DM101

Vazyme

Instruction for Use

Version 23.2

Vazyme

Contents

01/Product Description

The kit is intended for fast and easy genomic DNA (gDNA) isolation from 50 µl - 1 ml of fresh or frozen anticoagulated whole blood samples. This kit utilizes superparamagnetic particle paried with unique reagents for the effective removal of various impurities and specific adsorption of gDNA. The workflow does not require organic reagents such as phenol or chloroform, and highly purified gDNA can be obtained reproducibly within 1 h. The extracted gDNA can be directly used for downstream experiments, such as enzyme digestion, PCR, qPCR, library preparation, and Southern Blot.

02/Components

Components	DM101-01 (50 rxns)	DM101-02 (200 rxns)
Proteinase K	1 ml	4 ml
MagUltra Beads	1 ml	4 ml
Buffer DCL	20 ml	80 ml
Buffer WA	33 ml	2 × 66 ml
Buffer WB	10 ml	40 ml
Elution Buffer	20 ml	80 ml
Buffer EL	60 ml	240 ml

Proteinase K: Digest proteins in samples.
Buffer DCL: Release gDNA.
MagUltra Beads: Adsorb gDNA.
Buffer WA: Remove residual proteins and other impurities in gDNA.
Buffer WB: Remove residual salt ions in gDNA.
Elution Buffer: Elute gDNA from MagUltra Beads.
Buffer EL: Lyse red blood cells.

03/Storage

Store at 15 ~ 25°C and transport at room temperature.
If ambient temperatures often exceed 25°C, we suggest storing Proteinase K and MagUltra Beads at 2 ~ 8°C.

04/Applications

50 µl - 1 ml of non-nucleated fresh or frozen (EDTA/sodium citrate/sodium heparin) anticoagulated whole blood.

05/Self-prepared Materials

PBS, isopropanol, absolute ethanol, 1.5 ml or 2 ml Nuclease-free EP tubes;
Magnetic rack, vortex mixer, mini centrifuge, water bath.

06/Notes

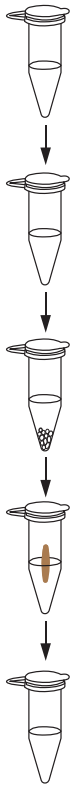
For research use only. Not for use in diagnostic procedures.

1. Mix blood samples thoroughly before extraction.
2. Before the first use, add the correct volumes of absolute ethanol to Buffer WA and Buffer WB according to the table below or as indicated on the label of the reagent bottle, and mix them well.

Reagent	DM101-01 Buffer WA	DM101-01 Buffer WB	DM101-02 Buffer WA	DM101-02 Buffer WB
Volume of Absolute Ethanol (ml)	44	40	88	160

3. Check if there is any precipitation in Buffer DCL and Buffer WA before use. If precipitates have formed, they can be re-dissolved in a 37℃water bath and mixed well before use.
4. Perform all steps at room temperature (15 ~ 25°C).

07/Mechanism & Workflow



Sample treatment:

a: If the sample volume is less than 200 µl, add to 200 µl with PBS.

b: If the sample volume is more than 200 µl and less than 1 ml, add Buffer EL (2 × the volumes of samples) , and mix by inversion.
Centrifuge at 10,000 rpm (11,500 × g) for 1 min and discard the supernatant. Add 50 µl of PBS and mix by vortexing.

Sample lysis:

◇ Add 20 µl of Proteinase K and 300 µl of Buffer DCL to the sample, and mix by vortexing for 30 sec.

◇ Incubate in a 65°C water bath for 5 min/15 min, mixing 3 times by inversion during incubation.

Nucleic acid purification:

◇ Lysis and binding: Add 20 µl of MagUltra Beads and 350 µl of isopropanol to the mixture, mix by vortexing for 5 min, and leave at room temperature for 2 min.

◇ Removal of proteins: Wash twice with Buffer WA.

◇ Removal of salt ions: Wash once with Buffer WB.

◇ Removal of ethanol: Discard the supernatant, and air dry at room temperature for 10 - 15 min.

Nucleic acid elution:

◇ Add 50 - 100 µl of Elution Buffer, and incubate in a 75°C water bath for 5 min to elute the gDNA.

08/Experiment Process

1. Sample lysis

1.1 Small sample volumes (50 - 200 µl):

a. Transfer 200 µl of the blood sample to a 1.5 ml centrifuge tube (Add to 200 µl with PBS if the sample volume is less than 200 µl).

b. Add 20 µl of Proteinase K and 300 µl of Buffer DCL, mix by vortexing for 30 sec, then incubate the centrifuge tube in a 65°C water bath for 5 min, mixing twice during incubation by inverting the tube 3 - 5 times each time.

c. Leave at room temperature for 5 min, and proceed to Step 2.

1.2 Large sample volumes (200 µl - 1 ml):

a. Add 2 × the sample volumes of Buffer EL to the blood sample and mix thoroughly by inverting the tube 10 times. Centrifuge at 10,000 rpm (11,500 × g) for 1 min and remove the supernatant, leaving the white blood cell pellet. Add 50 µl of PBS to the pellet, mix thoroughly by vortexing, and proceed to the next step.

b. Add 20 µl of Proteinase K and 300 µl of Buffer DCL, mix by vortexing for 30 sec, then incubate the centrifuge tube in a 65°C water bath for 15 min, mixing 3 times during incubation by inverting the tube 3 - 5 times each time.

▲ For large sample volumes, if there are still floccules after lysis at 65°C for 15 min, extend lysis time as appropriate until the solution is completely clear.

c. Leave at room temperature for 5 min, and proceed to Step 2.

2. Add 20 µl of MagUltra Beads and 350 µl of isopropanol to the above reaction system. Mix by vortexing for 5 min, then incubate at room temperature for 2 min.

▲ Mix the MagUltra Beads thoroughly by vortexing before use. To ensure the homogeneity of the beads during use, mix the beads by inversion after they are added to every 3 - 4 samples.

3. Centrifuge briefly, leave the centrifuge tube on the magnetic rack for 1 min until the beads are fully collected, and carefully remove the supernatant.

4. Remove the centrifuge tube from the magnetic rack. Add 700 µl of Buffer WA (check whether ethanol absolute has been added before use), and mix by vortexing for 1 min.

5. Centrifuge briefly, leave the centrifuge tube on the magnetic rack for 30 sec until the beads are fully collected, and carefully remove the supernatant.

6. Repeat Steps 4 and 5 once.

7. Remove the centrifuge tube from the magnetic rack. Add 700 µl of Buffer WB (check whether ethanol absolute has been added before use), and mix by vortexing for 1 min.

8. Centrifuge briefly, leave the centrifuge tube on the magnetic rack for 30 sec until the beads are fully collected, and carefully remove the supernatant.

▲ The residual liquid can be removed using a 10 µl pipette tip.

9. Keep the centrifuge tube on the magnetic rack. Uncap the tube and air dry at room temperature for 10 - 15 min.
   ▲ Ensure that ethanol is completely evaporated, as residual ethanol will interfere with subsequent enzymatic reactions. However, do not over-dry the beads to avoid interfering with elution.
10. Remove the centrifuge tube from the magnetic rack. Add 50 - 100 µl of Elution Buffer, mix by vortexing for 30 sec, then incubate the centrifuge tube in a 75°C water bath for 5 min, mixing twice during incubation by inverting the tube 3 - 5 times each time.
11. Centrifuge briefly, leave the centrifuge tube on the magnetic rack for 2 min until the beads are fully collected, and transfer the DNA solution to a new centrifuge tube.

09/FAQ & Troubleshooting

Question	Reasons	Solutions
Low DNA Yield	1. Improper storage, repeated freezing and thawing, or prolonged storage of blood samples	Use fresh blood samples for genomic DNA extraction.
	2. Too little/Too much blood sample used	Use the sample volume recommended in the Instructions for Use.
	3. Partial or complete activity loss of Proteinase K due to improper storage	Use new Proteinase K for digestion.
	4. Incomplete sample lysis	Ensure that the sample is thoroughly mixed with Proteinase K and Buffer DCL. Extend the lysis time in the 65°C water bath or mix more times by inversion as appropriate.
	5. Over-drying of beads	Inspect the beads during air-drying to avoid over-drying.
	6. 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.
	7. 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.
	8. Buffer WA or Buffer WB not supplemented with the correct volume of ethanol absolute	Add appropriate volumes of absolute ethanol as indicated on the label of the reagent bottle.
Low DNA Purity	1. Too little blood sample used	See above.
	2. Protein contamination	Buffer WA wash has been omitted, or only one wash has been performed. Wash twice with Buffer WA in accordance with the Instructions for Use.
	3. Salt ion contamination	Buffer WB wash has been omitted, or Buffer WB has not been supplemented with the correct volume of absolute ethanol. Add the correct volume of absolute ethanol as indicated on the label of the reagent bottle, and perform the wash step.
	4. Residual ethanol	The beads have not been properly dried. Extend the air-drying time as appropriate.
Special Samples	1. Cell-rich samples, such as the blood of leukemia patients or cord blood	Limit the input to 50 - 200 µl and extend lysis time (≥15 min) to ensure complete lysis.
	2. Cell samples	The input of cells should be less than 1×10^7.

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