Magnetic Universal Genomic DNA Kit

Cat. No.: D1372306 | Pack size: 50T | Storage: Room temperature

Overview

This kit uses superparamagnetic particles and optimized buffer solutions to isolate and purify high-quality genomic DNA from blood, dried blood spots, saliva, oral swabs, animal tissues, bacteria, cells, and FFPE samples. Under specific pH and salt ion conditions, the magnetic particles exhibit strong and specific affinity for nucleic acids. When the conditions are altered, the magnetic particles release the adsorbed nucleic acids, enabling rapid isolation and purification of nucleic acids. The entire operation process is safe and convenient. The extracted genomic DNA features large fragments, high purity, and stable and reliable quality, making it particularly suitable for automated extraction on high-throughput workstations. The DNA purified using this kit is applicable to various routine operations, including restriction enzyme digestion, PCR, quantitative real-time PCR, library construction, Southern blotting, microarray detection, and high-throughput sequencing.

Kit Contents & Storage

Cat. No.	Component	50T	Storage
D1372306A	Buffer MDA	30 mL	RT
D1372306B	Buffer GHL	20 mL	RT
D1372306C	Proteinase K	1.2 mL	RT
D1372306D	Buffer MW	68 mL	RT
D1372306E	Buffer EB	15 mL	RT
D1372306F	Magnetic Beads GF	0.55 mL	RT

Store at room temperature long term (15 months) under dry conditions.

Self-Prepared Reagents

RNase A, Lysozyme Solution.

Storage Conditions

This kit can be stored for 15 months at room temperature in a dry environment. If precipitation forms in the buffer, incubate the buffer in a 50 °C water bath for 10 minutes to redissolve the precipitate; this will not affect the extraction efficiency.

Pre-Experiment Preparation

Preparation of Isopropanol-Magnetic Beads GF Mixture (Taking the preparation volume for 10 samples as an example)

1) Add 3.3 ml of isopropanol [calculation: 0.3 × (10+1) = 3.3] to a centrifuge tube of appropriate capacity (the total volume after adding isopropanol and Magnetic Beads GF should be less than two-thirds of the centrifuge tube volume).

2) Add 110 µl of Magnetic Beads GF [calculation: 10 × (10+1) = 110] to the centrifuge tube from the previous step.

Note: *Vortex the magnetic beads for 20 seconds to mix them thoroughly before adding. Vortex the isopropanol-magnetic bead mixture for 20 seconds to obtain a homogeneous solution before use.*

Operating Procedures

I. Sample Pretreatment

A. Blood Samples (Anticoagulated Blood)

1. Pipette 250 µl of blood sample into a 1.5 ml centrifuge tube.

2. Add 20 µl of Proteinase K and 300 µl of Buffer GHL, vortex for 5 seconds to mix thoroughly. Place the centrifuge tube in a 75 °C water bath and incubate for 15 minutes, vortexing twice during incubation to remix.

3. Proceed to the manual purification and elution steps as described in Section II.

B. Blood Samples (Buffy Coat and Plasma-Depleted Samples)

1. Pipette 100–200 µl of buffy coat sample into a 1.5 ml centrifuge tube (equilibrate the sample to room temperature beforehand).

2. Add 20 µl of Proteinase K and 350 µl of Buffer GHL, invert the tube repeatedly to mix. Place it on a thermostatic shaking metal bath and lyse at 75 °C, 1,600 rpm for 15–30 minutes until no clumps are visible in the sample.

Note: *Buffy coat samples and plasma-depleted blood samples are relatively viscous with a high proportion of white blood cells. To ensure complete lysis, vortex the mixture thoroughly and appropriately extend the lysis time if necessary.*

3. Proceed to the manual purification and elution steps as described in Section II.

C. Large-Volume Blood Samples (2 ml Blood Sample as an Example)

1. Thaw the blood sample completely at room temperature. Add 2 ml of Buffer FL to the blood collection tube containing 2 ml of blood, invert 5 times to mix. Centrifuge at 3,600 rpm for 5 minutes, discard the supernatant. Add another 2 ml of Buffer FL, invert 5 times to mix, centrifuge again at 3,600 rpm for 5 minutes, discard the supernatant, retain 100–200 µl of Buffer FL in the tube, and vortex until the mixture is thoroughly homogenized.
2. Add 20 µl of Proteinase K and 350 µl of Buffer GHL, invert the tube repeatedly to mix. Place it on a thermostatic shaking metal bath and lyse at 75 °C, 1,600 rpm for 15–30 minutes until no clumps are visible in the sample.
3. Proceed to the manual purification and elution steps as described in Section II.

D. Dried Blood Spot Samples

1. Sample Processing: Place 3–10 pieces of 3×3 mm dried blood spot samples into a 1.5 ml centrifuge tube. Add 20 µl of Proteinase K, and simultaneously add Buffer MDA according to the ratio specified in the table below.

Table 2. Reagent Volume Requirements for Different Numbers of Dried Blood Spot Pieces

Number of Dried Blood Spot Pieces	Volume of Buffer MDA to Add
3	200 µL
5	300 µL
10	400 µL

Note: *For a large number of samples, Buffer MDA and Proteinase K solution can be pre-mixed in proportion. The mixture should not be stored for more than 1 hour, and it is best to prepare the mixture immediately before use.*

2. Vortex for 5 seconds, then place the tube in a preheated thermostatic shaker set at 75 °C, and incubate with shaking at 1,600 rpm for 45 minutes for lysis.

3. After lysis is completed, add 300 µl of Buffer GHL and vortex to mix thoroughly.
4. Proceed to the manual purification and elution steps as described in Section II.

E. Saliva Samples

1. Pipette 300 µl of saliva sample into a 1.5 ml centrifuge tube. Add 300 µl of Buffer GHL and 20 µl of Proteinase K, vortex for 5 seconds to mix thoroughly. Incubate at 75 °C for 15 minutes for lysis, vortexing twice during incubation to remix.

Note: *For a large number of samples, Buffer GHL and Proteinase K solution can be pre-mixed in proportion. The mixture should not be stored for more than 1 hour, and it is best to prepare the* mixture immediately before use.
2. Proceed to the manual purification and elution steps as described in Section II.

F. Oral Swab Samples

1. Sample Processing:

1) Dry swab samples: After sample collection, add 500 µl of Buffer MDA and 20 µl of Proteinase K, vortex for 10 seconds to mix thoroughly.

2) Swab samples with preservation solution: Pipette 300 µl of swab sample containing preservation solution into a 1.5 ml centrifuge tube. If the volume of preservation solution is insufficient, make up the volume to 300 µl with Buffer MDA. Add 20 µl of Proteinase K and vortex for 10 seconds to mix thoroughly.
2. Incubate at 75 °C for 15 minutes for lysis, vortexing twice during incubation to remix.
3. After lysis is completed, add 300 µl of Buffer GHL and vortex to mix thoroughly.
4. Proceed to the manual purification and elution steps as described in Section II.

G. Tissue Samples

1. Weigh 10–50 mg of animal tissue, cut it into small pieces as much as possible. Add 300 µl of Buffer MDA and 20 µl of Proteinase K, and homogenize with an electric homogenizer until the tissue is fully lysed.

1) For fully homogenized samples, the 65 °C digestion step can be omitted.

2) For samples with visible tissue clumps, it is recommended to incubate at 65 °C for 30 minutes until complete digestion.

3) For mouse tail samples, incubate at 56 °C overnight for digestion.

4) For hair with hair follicles and feather shaft samples, supplement with 20 µl of 1 M DTT (self-prepared) and incubate for 60 minutes to overnight for digestion.

Note: *After complete sample digestion, if there are tissue debris, centrifuge at 12,000 rpm for 1 minute to remove residual impurities. If RNA removal is required, add 4 µl of RNase A, vortex to mix thoroughly, and incubate at room temperature for 5–10 minutes.*

2. Transfer 300 µl of the processed sample solution into a new 1.5 ml centrifuge tube.
3. Add 300 µl of Buffer GHL and vortex to mix thoroughly.
4. Proceed to the manual purification and elution steps as described in Section II.

H. Bacterial Samples

1. Pipette 1–5 ml of bacterial culture solution, centrifuge at 12,000 rpm for 1 minute, and discard the supernatant.

2. Add 300 µl of Buffer MDA and 20 µl of Proteinase K to the bacterial pellet, and vortex until the bacteria are completely resuspended.

Note: *For Gram-positive bacteria that are difficult to lyse, step 2 can be skipped, and lysozyme solution can be added for cell wall disruption. The specific method is as follows: Add 110 µl of buffer solution (20 mM Tris, pH 8.0; 2 mM Disodium EDTA; 1.2% Triton X-100) and 70 µl of lysozyme solution, and incubate at 37 °C for more than 30 minutes.*

For bacterial extraction from sputum samples:

1) Add 1 M NaOH solution (self-prepared) to the sputum sample at a 1:1 volume ratio and incubate for 30 minutes for liquefaction.

2) Centrifuge at 4,700 rpm for 5 minutes and discard the supernatant.

3) Add 300 µl of Buffer MDA, vortex until the pellet is completely resuspended. Heat at 95 °C for 10 minutes for lysis and then cool to room temperature.

3. Add 300 µl of Buffer GHL and 20 µl of Proteinase K to the suspension from the previous step, vortex for 10 seconds to mix thoroughly. Incubate at 75 °C for 15 minutes for lysis until the bacterial solution becomes clear; if necessary, extend the incubation time until the solution is clear.

Note: *If RNA removal is required after the above steps, add 4 µl of RNase A (100 mg/ml) (Catalog No.* R665521), vortex to mix thoroughly, and incubate at room temperature for 5–10 minutes.

4. Proceed to the manual purification and elution steps as described in Section II.

I. Cell Samples

1. Resuspend the cell pellet containing approximately 1×10^6–10^7 cells thoroughly by vortexing with 300 µl of Buffer MDA and 20 µl of Proteinase K.

2. Add 300 µl of Buffer GHL, vortex for 10 seconds to mix thoroughly. Incubate at 75 °C for 15 minutes for lysis until the cell sample becomes clear; if necessary, extend the incubation time until the sample is clear.

Note: *If RNA removal is required after the above steps, add 4 µl of RNase A (100 mg/ml) (Catalog No. R665521), vortex to mix thoroughly, and incubate at room temperature for 5–10 minutes.*

3. Proceed to the manual purification and elution steps as described in Section II.

J. FFPE Samples

1. Sample Processing:

a. Place 2–8 paraffin sections (10 µm thick, 1×1 cm^2 in size) into a 1.5 ml sterile centrifuge tube. Add 300 µl of environment- friendly dewaxing oil DO (self-prepared), 300 µl of Buffer MDA and 20 µl of Proteinase K respectively, vortex vigorously for 10 seconds, and incubate at 75 °C for 30–60 minutes for digestion until the tissue clumps disappear.

b. Incubate at 90 °C for 1 hour for further digestion.

2. Transfer 300 µl of the lower-layer solution into a new 1.5 ml centrifuge tube for subsequent experiments.

3. Add 300 µl of Buffer GHL to the digested sample and vortex to mix thoroughly.

4. Proceed to the manual purification and elution steps as described in Section II.

II. Manual Purification and Elution Steps

1. Add 310 µl of thoroughly mixed isopropanol-Magnetic Beads GF mixture, vortex for 5 seconds to homogenize. Then place the centrifuge tube on a thermomixer at 25 °C, 1,600 rpm and shake for 10 minutes, or invert the tube continuously for 10 minutes to mix well.

2. Place the centrifuge tube on a magnetic rack and let it stand for 1 minute. After the magnetic beads are completely adsorbed, discard the solution thoroughly (keep the centrifuge tube fixed on the magnetic rack).

3. Remove the centrifuge tube from the magnetic rack, add 750 µl of Buffer MW, then either vortex in pulses for 1 minute, or vortex for 5 seconds followed by shaking on a thermomixer at 25 °C, 1,600 rpm for 2 minutes (ensure the magnetic beads remain suspended during shaking). Place the tube back on the magnetic rack and let it stand for 1 minute. Once the beads are fully adsorbed to the tube wall, gently invert the magnetic rack to wash off impurities on the tube cap, then discard the solution completely (keep the tube fixed on the rack).

4. Repeat Step 3.

5. Remove the centrifuge tube from the magnetic rack, add 750 µl of 75% ethanol (self-prepared). Then either vortex in pulses for 1 minute, or vortex for 5 seconds followed by shaking on a thermomixer at 25 °C, 1,600 rpm for 2 minutes (ensure the magnetic beads remain suspended during shaking). Place the tube back on the magnetic rack and let it stand for 1 minute. Once the beads are fully adsorbed to the tube wall, gently invert the magnetic rack to wash off impurities on the tube cap, then discard the solution completely (keep the tube fixed on the rack).

6. Repeat Step 5.

7. Keep the centrifuge tube fixed on the magnetic rack, use a pipette to remove any residual solution from the tube bottom and cap, then air-dry at room temperature for 5–10 minutes to allow complete ethanol evaporation.

8. Remove the centrifuge tube from the magnetic rack, add 50–200 µl of Buffer EB. Vortex to resuspend the magnetic beads completely in the eluent, then either incubate on a thermomixer at 56 °C, 1,600 rpm for 10 minutes for elution, or place the tube in a 56 °C water bath for 10 minutes with vortexing for 10 seconds every 3 minutes during incubation.

9. Place the centrifuge tube on the magnetic rack and let it stand for 3 minutes. After the magnetic beads are fully adsorbed to the tube wall, use a pipette to transfer the eluent to a new centrifuge tube and store at -20 °C until use.

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