

Magnetic Bead Method Saliva DNA Extraction Kit

Project number: M666121

Storage conditions: room temperature.

Product Content:

Component	M666121 96preps
BufferKCL	80mL
BufferGW1 (concentrate)	80mL
BufferGW2 (concentrate)	26mL
BufferMW3	80mL
BufferEB	30mL
ProteinaseK	2×25mg
ProteinaseKStorageBuffer	2×1.25mL
MagbeadsPN	2×1mL

Product Introduction:

This kit provides a simple, rapid and efficient method for extracting genomic DNA from saliva. In the presence of high salt, DNA is bound to the surface of silica-coated Magbeads. After rinsing, the high-purity DNA is eluted in Buffer EB or deionized water. The purified DNA has good purity (A260/280 ratio between 1.7 and 1.9) and high integrity (>15kb), and can be used for downstream experiments such as second-generation sequencing, quantitative PCR, and microarray detection.

Bring your own instruments and reagents

- 1) Thermostatic homogenizer
- 2) 2/15 ml magnetic holder
- 3) 32-channel nucleic acid extractor
- 4) 96-channel nucleic acid extractor
- 5) 96 DW Plate
- 6) 8channelComb
- 7) Spintips pack
- 8) Anhydrous ethanol

Pre-experiment Preparation and Important Notes

1. Add 1.25 ml of Proteinase K Storage Buffer to 25 mg of Proteinase K to dissolve it, and then store it at -20°C. Do not leave the prepared Proteinase K solution at room temperature for a long time and avoid repeated freezing and thawing to avoid affecting its activity.
2. Add anhydrous ethanol to Buffer GW1 and Buffer GW2 according to the labels of the reagent bottles and label them properly before the first use.

3. Freezing and centrifugation of Magbeads is strictly prohibited. Freezing and centrifugation may cause irreversible damage to Magbeads.

procedure

I. Manual single-tube operation

1. Sequentially add 20 μ l of Proteinase K, 350 μ l of saliva and 500 μ l of BufferKCL to a 2.0 mL centrifuge tube.

2. After vortexing and mixing, put the centrifuge tube on a thermostatic mixer at 65°C and 1600 rpm for 20 minutes to form the lysate.

Note: If a thermostatic mixer is not available, vortex the centrifuge tube for 10 seconds and incubate in a 65° C water bath for 20 minutes at 5-minute intervals. The clock vortex oscillates for 10 seconds.

3. Remove the centrifuge tube from the thermomixer, centrifuge briefly and add 20 μ l of MagbeadsPN. Afterwards, place the tube on a thermomixer at 25° C and 1600 rpm for 10 minutes of shaking and lysing or mix the tube for 15 minutes of continuous inversion.

4. Place the centrifuge tube on the magnetic rack and let it stand for 1 minute until the Magbeads are completely adsorbed to the sidewalls of the tube and the solution is discarded completely (keep the tube fixed on the magnetic rack).

5. Remove the centrifuge tube from the magnetic rack, add 750 μ l of Buffer GW1 (check that anhydrous ethanol has been added before use) and vortex for 1 minute or vortex for 5 seconds, then place the tube on a thermostatic mixer at 25° C and 1600 rpm for 2 minutes (make sure that the Magbeads are in the homogenized state during the vortexing process). Afterwards, place the tube on a magnetic rack for 1 minute and gently invert the rack to remove any impurities from the cap and discard the solution (keep the tube fixed on the magnetic rack).

6. Repeat step 5.

7. Remove the centrifuge tube from the magnetic rack, add 750 μ l of Buffer GW2 (check that anhydrous ethanol has been added prior to use) and vortex for 1 minute or vortex for 5 seconds, then place the tube on a thermostatic mixer at 25° C and 1600 rpm for 2 minutes (make sure that the Magbeads are in the homogenized state during the vortexing process). Afterwards, place the tube on a magnetic rack for 1 minute and gently invert the rack to remove any impurities from the cap and discard the solution (keep the tube fixed on the magnetic rack).

8. Keeping the centrifuge tube fixed on the magnetic rack, add 750ul of Buffer MW3 to the centrifuge tube and discard the solution as soon as the suspended beads are re-attached to the magnetic rack.

9. Keeping the centrifuge tubes fixed on a magnetic rack, use a pipette to further remove the solution from the bottom and cap of the centrifuge tubes and leave them at room temperature for 5-10 minutes to allow the ethanol to evaporate.

10. Remove the centrifuge tube from the magnetic rack and add 50-200 μ l of BufferEB. Vortex the beads to completely suspend them in the eluent and elute by shaking on a thermostatic mixer at 56° C, 1600 rpm for 10 minutes or incubate the tube in a water bath at 56° C for 10 minutes, vortexing for 10 seconds at 3-minute intervals.

11. Place the centrifuge tube on a magnetic rack and let it stand for 2 minutes. When the Magbeads are completely adsorbed to the side wall of the centrifuge tube, use a pipette to transfer the eluate to a new centrifuge tube and store it at -20°C.

II. Matching with 32-channel nucleic acid extractor

1. Add the appropriate reagents to the 96DW deep well plate according to the following table

Position	Reagent
1&7 Colume	ProteinaseK:20μl Sample:350μl BufferKCL:500μl MagbeadsPN:20μl
2&8 Colume	Buffer GW1: 750 μl
3&9 Colume	Buffer GW1: 750 μl
4& 10 Colume	Buffer GW2: 750 μl
5& 11 Colume	Buffer MW3: 750 μl
6& 12 Colume	Buffer EB: 100 μl

4. Put the reagent-added deep-well plate and magnetic sleeve in the corresponding position of the 32-channel nucleic acid extractor, run the saliva extraction program, and take out the deep-well plate and magnetic sleeve about 28 minutes after the program is finished.

5. Transfer the elution products in columns 6&12 of the deep-well plate to a 1.5 ml centrifuge tube for cryopreservation.

III. Matching with 96-channel nucleic acid extractor

1. Add the appropriate reagents to the 96DW deep well plate according to the following table

Position	Reagent
Plate 1	ProteinaseK:20μl Sample:350μl BufferKCL:500μl MagbeadsPN:20μl
Plate 2	BufferGW1: 750 μl
Plate 3	BufferGW1: 750 μl
Plate4	BufferGW2: 750 μl

Plate 5	BufferMW3: 750 μ l
Plate 6	Buffer EB: 100 μ l

- Put the reagent-added deep-well plate and magnetic sleeve in the corresponding position of the 96-channel nucleic acid extractor, run the saliva extraction program, and take out the deep-well plate and magnetic sleeve after about 28 minutes.
- Transfer the elution product in Plate6 to a 1.5 ml centrifuge tube for cryopreservation.