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Fast Magbead Free-Circulating DNA Maxi Kit

Product Number: DNK53

Shipping and Storage

Magbeads ZN 2-8℃, other components at room temperature (10-30℃).

Component

Component	DNK53-48T
Buffer CTL	2×72mL
Buffer CTW	60mL
Buffer GCW2 （concentrate）	2×10mL
RNase-Free Water	10mL
Proteinase K	5mL
Magbeads ZN	2×1mL

Description

This kit is suitable for the purification and recovery of free circulating/cell-free DNA from cell-free body fluids such as plasma, serum, and urine (1-5 mL). The kit combines lysis at room temperature with nucleic acid adsorption into a single step, eliminating the need for ice baths or pauses when adding binding buffer and magnetic beads, significantly simplifying the workflow. In the presence of high salt, free DNA binds to the surface of silica-coated magnetic beads. After washing, free DNA is eluted in enzyme-free water. The yield of free DNA is highly dependent on sample type, storage conditions, duration, and inter-individual variations. The kit features high extraction efficiency, excellent reproducibility, simple and efficient operation, and rapid processing. The purified free DNA is stable and reliable in quality, suitable for downstream routine experiments such as quantitative PCR and second-generation sequencing library construction.

Self provided instruments and reagents

1. **Manual single tube operation**
   - 1.1. Vortex oscillator
   - 1.2. Constant temperature mixer
   - 1.3. 16 hole magnetic bracket (1.5mL/2mL)
   - 1.4. Anhydrous ethanol (AR analytical grade)
2. **Match with nucleic acid extractor**
   - 2.1. Fully automatic nucleic acid extractor
   - 2.2. 24 DW Plate and Tips Pack V2
   - 2.3. Anhydrous ethanol (AR analytical grade)

Preparation and important precautions before the experiment

1. Before the first use, anhydrous ethanol should be added to Buffer GCW2 according to the instructions on the reagent bottle label.
2. Freezing and high-speed centrifugation are strictly prohibited for Magbeads ZN, otherwise it may cause irreversible damage to Magbeads ZN. Please shake Magbeads ZN thoroughly for 30 seconds before each use to mix evenly.
3. Before use, please check whether there is crystallization or precipitation in Buffer CTL and Buffer CTW. If there is crystallization or precipitation, it can be restored to clarity in a water bath at 50℃ for a few minutes.
4. Fresh samples should be processed or packaged as soon as possible and frozen at -70℃ to avoid repeated freeze-thaw cycles. After thawing, the frozen samples should be centrifuged at 2000 × g for 1 minute and the supernatant should be taken for

experimentation.

Protocol

1. Manual, taking 2mL of plasma as an example

1.1. Add 100μL Proteinase K, 2mL plasma, 3mL Buffer CTL, and 30μL Magbeads ZN to the centrifuge tube in sequence. Please refer to the attached table for other sample volumes.

Note: To avoid inactivation of Proteinase K, please add the reagents in sequence. Do not directly add Buffer CTL to Proteinase K solution.

1.2. After thoroughly mixing the sample and reagent, place them on a constant temperature mixer and shake at room temperature of 1200 rpm for 20 minutes, or place them on a disc mixer and mix at 70 rpm/min for 20 minutes to keep the magnetic beads suspended and fully bound to the nucleic acid. After incubation, centrifuge briefly to remove droplets from the inner wall of the tube.

1.3. Place the centrifuge tube on the magnetic rack and let it stand for 2 minutes. Wait for the magnetic beads to adsorb onto the magnetic rack. After the solution inside the tube becomes clear, flip the centrifuge tube and rinse the residual magnetic beads on the bottle cap. Leave it for about 1 minute, then discard the solution.

1.4. Add 750μL Buffer CTW, vortex for 30 seconds to fully suspend the magnetic beads, and briefly centrifuge to remove droplets from the inner wall of the tube cap.

Note: All liquids and magnetic beads from step 4 can be transferred to a 2mL centrifuge tube for subsequent operations. If there are residual magnetic beads on the tube wall, rinse with 100μL Buffer CTW and transfer to a centrifuge tube.

1.5. Fix the centrifuge tube on the magnetic frame and let it stand for 1 minute. When all the magnetic beads are adsorbed, carefully discard the solution.

1.6. Add 750μL Buffer GCW2 (please check if anhydrous ethanol has been added before use), vortex for 30 seconds, and briefly centrifuge to remove droplets from the inner wall of the tube cap.

1.7. Fix the centrifuge tube on the magnetic frame and let it stand for 1 minute. When all the magnetic beads are adsorbed, carefully discard the solution.

1.8. Repeat steps 6-7.

1.9. Fix the centrifuge tube on the magnetic frame, open the lid and let it dry at room temperature for 5-10 minutes.

Note: Ethanol residue can inhibit enzymatic reactions, so ensure that ethanol evaporates completely when drying, and wait for the surface of the magnetic beads to become matte and not dry or crack. But do not excessively dry the magnetic beads to avoid difficulty in elution.

1.10. Remove the centrifuge tube from the magnetic frame, add 50-100μL RNase Free Water to the centrifuge tube, vortex and oscillate to fully suspend the magnetic beads in the eluent, and then fix the centrifuge tube on a constant temperature mixer at 1600 rpm for 10 minutes to oscillate and elute.

1.11. Fix the centrifuge tube on the magnetic frame and let it stand for 2 minutes. After the magnetic beads are completely adsorbed on the side wall of the centrifuge tube, transfer the eluent to a new centrifuge tube using a pipette and store it at -20 ± 5℃ for later use.

2. Matching with the fully automatic nucleic acid extractor, taking 2mL and 4mL samples as examples

2.1. Add reagents to the 24 DW deep hole plate according to the table below:

Sample volume	2 mL	4 mL
Position	Reagent and dosage	Reagent and dosage
Plate 1	Proteinase K：100μL	Proteinase K：200μL
	Sample: 2mL	Sample: 3mL
	Buffer CTL：3mL	Buffer CTL：6mL
Plate 2	Buffer CTW: 0.75mL	Buffer CTW: 1.5mL
Plate 3	Buffer GCW2: 0.75mL	Buffer GCW2: 1.5mL
	Magbeads ZN：30μL	Magbeads ZN：60μL

Plate 4	Buffer GCW2: 0.75mL	Buffer GCW2: 1.5mL
Plate 5	RNase-Free Water: 100μL	RNase-Free Water: 100μL

2.2. Place the deep hole plate in the corresponding position, place the magnetic sleeve tips on Plate 3, and run the extraction program.

2.3. After about 55 minutes, the program runs and ends. Transfer the DNA elution product from "Plate 5" to a centrifuge tube and store it at -20 ± 5℃ for future use.

Appendix: Dosage of reagents for different sample volumes (please add the solution in order during lysis and binding)

Step	Sample volume	1mL	2mL	3mL	4mL	5mL
Cracking and binding	Proteinase K	50μL	100μL	150μL	200μL	250μL
	Buffer CTL	1.5mL	3mL	4.5mL	6mL	7.5mL
	Magbeads ZN	20μL	30μL	45μL	60μL	75μL
Rinse	Buffer CTW	0.5mL	0.75mL	1.1mL	1.5mL	1.5mL
	Buffer GCW2	0.5mL	0.75mL	1.1mL	1.5mL	1.5mL
	Buffer GCW2	0.5mL	0.75mL	1.1mL	1.5mL	1.5mL