MagicPure® Cell-Free DNA Kit II

Please read the datasheet carefully prior to use

Cat. No. EC211

Storage: Magnetic Cell-Free Beads at 2-8°C for one year (avoid freezing); others at room temperature (15-25°C) for one year.

Description

This kit lyses samples by enzymatic hydrolysis, and efficiently purifies cell-free DNA by specific adsorption of silica magnetic beads. It is suitable for isolating and purifying high quality cell-free DNA from 0.5-10 ml serum or plasma. The extracted DNA can be used for PCR, qPCR, NGS, *etc.* It is compatible with high-throughput magnetic rob-based nucleic acid extractor.

Highlights

- Simple operation and fast extraction with no centrifugation required.
- High yield and high purity.

Kit Contents

Component	EC211-01/11 (50 rxns)
Lysis Buffer 36 (LB36)	10 ml
Binding Buffer 36 (BB36)	125 ml
Clean Buffer 36 (CB36)	66 ml
Wash Buffer 36 (WB36)	20 ml
Elution Buffer 36 (EB36)	4 ml
Proteinase K (20 mg/ml)	4×1 ml
Magnetic Cell-Free Beads	2 ml
Magnetic Stand (16 hole)	1 pc/-

Starting material

- Serum and plasma storage: at 2-8°C, up to 4 hours for short term; at -80°C for long-term storage.
- Avoid repeated freeze-thaw cycles.

Procedures

- Before starting, add 34 ml isopropyl alcohol to CB36 and add 80 ml anhydrous alcohol to WB36.
- The user needs to prepare magnetic stands for 15 ml or 50 ml centrifuge tubes as required.
- All the magnetic separations need to be performed at room temperature. Vortex the magnetic beads before use.

1. According to the volume of the sample, add reagents and the sample to a centrifuge tube in the order provided in the table below.

Reagents	Plasma Volume				
	0.5 ml	1 ml	2 ml	4 ml	10 ml
Proteinase K	20 µl	40 µl	80 µl	160 µl	400 µl
Plasma sample	0.5 ml	1 ml	2 ml	4 ml	10 ml
LB36	50 µl	100 µl	200 µl	400 µl	1000 µl

2. Vortex for 15 seconds, and incubate at 60°C for 20 minutes (Mix by inversion 2-3 times during the incubation).
3. Place the tube on ice for 3 min. Add reagents in the order provided in the table below.

Component	Plasma Volume				
	0.5 ml	1 ml	2 ml	4 ml	10 ml
Binding Buffer 36	625 µl	1.25 ml	2.5 ml	5 ml	12.5 ml
Magnetic Cell-Free Beads	10 µl	20 µl	40 µl	80 µl	200 µl

4. Vortex for 15 seconds, and incubate at room temperature for 10 minutes (Mix by inversion 2-3 times during the incubation).
5. Place the centrifuge tube on a fitted magnetic stand for magnetic separation
   Recommendations for magnetic separation: Put the centrifuge tubes on the magnetic stand, and twirl the tube left and right gently. Invert the magnetic stand gently 2-3 times when the magnetic beads gather on the tube wall close to the magnetic stand, to make the beads on the lid also gather on the tube wall. Then let the tube stand for 2 minutes, so that the beads will be adsorbed to the tube wall thoroughly.
6. Pipette and discard the supernatant from the tube wall at the opposite side of magnetic beads. Do not pipette the beads. Take the tubes off the magnetic stand, and add 1 ml CB36 (Please check if isopropyl alcohol has been added before use). Vortex for 15 seconds, and then perform magnetic separation on a fitted magnetic stand.
   If a 15 ml or 50 ml centrifuge tube has been used, transfer the suspension of CB36 and magnetic beads to a new 1.5 ml centrifuge tube for magnetic separation. If there are still some residual beads in the 15/50 ml centrifuge tube, transfer the supernatant from the 1.5 ml centrifuge tube back to the 15/50 ml tube after magnetic separation. Wash the tube with the supernatant and transfer the suspension to the 1.5 ml centrifuge tube for another magnetic separation.
7. Discard the supernatant. Take the tubes off the magnetic stand, and add 1 ml CB36. Vortex for 15 seconds and perform magnetic separation on a magnetic stand.
8. Discard the supernatant. Take the tubes off the magnetic stand, and add 1 ml WB36 (Please check if isopropyl alcohol has been added before use). Vortex for 15 seconds, and then perform magnetic separation on a magnetic stand.
9. Repeat Step 8 once.
10. Discard the supernatant, including the liquid on the lid. In order to remove the supernatant thoroughly, we suggest the user to use smaller size pipette tips to pipet again.
11. Leave the tube on the magnetic stand and air dry at room temperature for 5-10 minutes.
12. Remove the tube from the magnetic stand. Add EB36 according to the plasma volume and the table below. Vortex vigorously for 5 minutes.

Component	Plasma Volume				
	0.5 ml	1 ml	2 ml	4 ml	10 ml
EB36	20 µl	30 µl	50 µl	75 µl	200 µl

13. Put the centrifuge tube on the magnetic stand for magnetic separation. Transfer the supernatant to a new 1.5 ml centrifuge tube (It is recommend to use low nucleic acid binding centrifuge tube). Avoid pipetting the beads.
14. Store the DNA at -20°C.

Notes

- The reaction times of the kit is calculated based on 2 ml plasma.
- Avoid repeated freeze-thaw cycles to ensure high quality of the extracted DNA.
- Use sterile, nucleic-acid-free and nuclease-free centrifuge tubes and pipette tips.
- The amount of cell-free DNA is extremely low, so we recommend the user to use low nucleic acid binding centrifuge tubes for plasma storage, DNA extraction and DNA storage.
- Make sure to mix well the magnetic beads by vortexing before use.

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