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EasyMag cfDNA Purification Kit from serum/plasma

Catalog Number: D112-1, D112-2

Table 1. Kit Components and Storage

Kit Component	D112-1 (50 preps)	D112-2 (200 preps)	Storage	Stability
Buffer ACL	50 mL	2×100 mL	RT	The product is stable for one year when stored as directed.
Buffer ACB*	50 mL	3×60 mL	RT	
Buffer ACW1*	15 mL	53 mL	RT	
Buffer ACW2*	10 mL	25 mL	RT	
Buffer AE	15 mL	30 mL	RT	
Proteinase K (20 mg/mL)	2×1.3 mL	10 mL	-20℃	
Carrier RNA (0.2 mg/mL)	250 µL	1 mL	-20℃	
MagBinding Beads	2.5 mL	10 mL	RT	

***** Prior to use, add isopropanol to **Buffer ACB** according to the bottle label; add absolute ethanol to **Buffer ACW1, Buffer ACW2** according to the bottle label.

Product Description

EasyMag cfDNA Purification Kit provides a rapid and reliable isolation of circulating DNA from plasma, serum, and other cellular body fluids. The kit can be processed manually or on an automated platform. The uniquely formulated lysis and binding buffers allow samples to be completely lysed, and binded to magnetic beads. The impurities such as cellular debris, hemoglobin, and other proteins are washed away using washing buffer. The isolated circulating DNA is ready for applications such as PCR, microarrays, and next generation sequencing.

Features

- ❖ Fast – DNA purification process in less than 2 hr.
- ❖ Safe – No Phenol/chloroform extractions.
- ❖ High-quality – DNA is suitable for a variety of downstream applications.

Purification Protocol

Note: The following protocol is designed for 1 mL plasma or serum samples. The purification method can be scaled up or down accordingly by adjusting the buffer volumes of Buffer ACL and Buffer ACB.

1. Transfer 50 µL Proteinase K solution to a clean 5 mL centrifuge tube.
2. Add 1 mL of serum or plasma to the 5 mL tube.
3. Add 0.8 mL Buffer ACL/Carrier RNA (1 µg) to the sample, and vortex for 30 seconds.

   Note: Buffer ACL may be precipitated during storage, if happen, heat it at 50^{o}C to dissolve. Premix Buffer ACL with Carrier RNA, the amount of Carrier RNA for each sample is 1 µg (5 µL). In order to ensure efficient lysis, it is essential that the sample and Buffer ACL are mixed thoroughly to yield a homogeneous solution.

4. Incubate at 60°C for 30 min. Vortex briefly once during incubation.
5. Add 1.8 mL Buffer ACB to the lysate in the tube, and vortex for 30 seconds.

   Note: Buffer ACB must be diluted with isopropanol according to the bottle label before use.
6. Incubate the lysate-Buffer ACB mixture in the tube for 5 min on ice.
7. Add 50 µL MagBinding Beads to the lysate-Buffer ACB mixture, and vortex at maximum speed for 15-20 seconds.
8. Incubate with shaking at RT for 5 min.
9. Transfer the tube to a magnetic rack for an additional 2 min or until beads pellet and supernatant is cleared. With the tube on the magnetic rack remove the supernatant and discard.
10. Add 600 µL of Buffer ACW1 to the tube. Resuspend the beads by pipetting up and down or vortexing at 1,500 rpm for 30 seconds. Replace the tube on the magnetic rack for 2 min or until beads pellet. Remove and discard the supernatant.

    Note: Buffer ACW1 must be diluted with absolute ethanol according to the bottle label before use.
11. Add 600 µL of Buffer ACW2 to the tube. Resuspend the beads by pipetting up and down or vortexing at 1,500 rpm for 30 seconds. Replace the tube on the magnetic rack for 2 min or until beads pellet. Remove and discard the supernatant.

    Note: Buffer ACW2 must be diluted with absolute ethanol according to the bottle label before use.
12. Add 700 µL of ethanol (95-100%) to the tube. Resuspend the beads by pipetting up and down or vortexing at 1,500 rpm for 30 seconds. Replace the tube on the magnetic rack for 2 min or until beads pellet. Remove and discard the supernatant.
13. Leave the tube on the magnetic rack, open the cap and air dry the beads at RT for 10-15 min.

    Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.
14. Add 20-50 µL Buffer AE to the tube, resuspend the beads by vortexing for 1 min. Incubate with shaking at RT for 4 min.
15. Replace the tube on the magnetic rack for 2 min or until beads pellet. Transfer the cleared supernatant to a clean tube.
16. Store the DNA at -20ºC.

Troubleshooting

Problem	Possible cause and suggestions
Low yield	• Sample old or frozen and thawed more than once: Repeated freezing and thawing should be avoided as this may lead to DNA degradation. Always use fresh samples or samples thawed only once. • Buffer ACB prepared incorrectly: Buffer ACB must be diluted with isopropanol before use. • Improper washing: Buffer ACW1, buffer ACW2 must be diluted with absolute ethanol before use. • Sample has low DNA content: Increase starting material and volume of all reagents proportionally.
Poor performance in downstream applications	• Salt contamination: Repeat Buffer ACW2 wash twice. • Ethanol contamination: Dry the beads at 50°C for 10 min.