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EasySC cfDNA Purification Kit from serum/plasma

Catalog Number: D111-1, D111-2

Table 1. Kit Components and Storage

Kit Component	D111-1 (50 preps)	D111-2 (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℃	
Mini Column	50	200	RT	
2 mL Collection Tube	50	200	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

EasySC cfDNA Purification Kit from serum/plasma provides a rapid and easy method for the isolation of circulating DNA from plasma, serum, and other cellular body fluids. The kit allows for simultaneous processing of single or multiple samples in under 2 hours. EasySC cfDNA Purification Kit uses a specially formulated buffer system to lyse sample under denaturing conditions, then the lysis was transferred to the DNA column where DNA binds to column matrix and cellular debris, hemoglobin, and other proteins are washed away. 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. Connect a DNA Mini Column to a vacuum manifold. Carefully apply the lysate-Buffer ACB mixture from step 6 into the DNA Mini column. Switch on the vacuum pump. When all lysates have been drawn through the columns completely, switch off the vacuum pump.
8. Insert the DNA Mini Column into a 2 mL Collection Tube. Add 600 µL of Buffer ACW1 to the DNA Mini Column, invert and mix once, then centrifuge at 10,000 x g for 1 min.

   Note: Buffer ACW1 must be diluted with absolute ethanol according to the bottle label before use.
9. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer ACW2 to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min.

   Note: Buffer ACW2 must be diluted with absolute ethanol according to the bottle label before use.
10. Discard the filtrate and reuse the collection tube. Add 700 µL of ethanol (95-100%) to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min.
11. Discard the filtrate and reuse the collection tube. Centrifuge the empty DNA Mini Column at 12,000 x g for 3 min.

    Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.
12. Transfer the DNA Mini Column into a clean 1.5 mL microcentrifuge tube. Open the lid, and incubate the assembly at 56°C for 10 min to dry the membrane completely.
13. Place the DNA Mini column in a clean 1.5 ml microcentrifuge tube. Add 20-50 µL Buffer AE to the center of the membrane. Close the lid and incubate at RT for 3 min, then centrifuge at 10,000 x g for 1 min.

    Note: To improve the yield, repeat this step for a second elution step.
14. Discard the column and 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: incubate the column at 56°C for 10 min to dry the membrane completely.