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EasySC DNA Purification Kit from blood

Catalog Number: D109-1, D109-2

Table 1. Kit Components and Storage

Kit Component	D109-1 (50 preps)	D109-2 (200 preps)	Storage	Stability
Buffer AL	15 mL	60 mL	RT	The product is stable for one year when stored as directed.
Buffer DW1*	15 mL	53 mL	RT	
Buffer DW2*	15 mL	2×25 mL	RT	
Buffer AE	15 mL	30 mL	RT	
Mini Column	50	200	RT	
2 mL Collection Tube	50	200	RT	

***** Prior to use, add absolute ethanol to **Buffer DW1, Buffer DW2** according to the bottle label.

Product Description

EasySC DNA Purification Kit from blood provides rapid total DNA isolation from 10-250 µL of fresh and frozen anticoagulated whole blood. This kit can also be used for the preparation of genomic DNA from buffy coat, serum, plasma, bone marrow, lymphocytes, platelets, and other body fluids. This kit allows for simultaneous processing of single or multiple samples in less than 30 minutes. Phenol/chloroform extraction, and time-consuming steps such as precipitation with isopropanol or ethanol have been eliminated. The isolated DNA is ready for applications such as PCR, Southern blotting, or restriction enzyme digestion.

Features

- Fast – DNA purification process in less than 30 min.
- Safe – No Phenol/chloroform extractions.
- High-quality – DNA is suitable for a variety of downstream applications.

Purification Protocol

1. Transfer 250 µL fresh or frozen anticoagulated whole blood, serum, plasma, or other body fluids to a clean 1.5 mL microcentrifuge tube. If the sample volume is less than 250 µL, bring the volume up to 250 µL with PBS buffer, or Buffer AE (provided).

   Note: When processing coagulated blood samples, homogenize the samples with a mechanical or glass homogenizer to fully liquefy them before extracting. Since red blood cells of non-mammalian animals such as birds and fish are nucleated, their DNA content is extremely rich, and the kit can only process 5-20 µL of blood at a time.

2. Add 250 µL Buffer AL to the sample. Vortex at maximum speed for 15-20 seconds, and incubate at 65°C for 15-30 minutes. Vortex briefly once during incubation.

   Note: Buffer AL may be precipitated during storage, if happen, heat it at 50^{o}C to dissolve. If RNA need be removed, add 10 µL RNase A Solution (10 mg/mL) to the sample.

3. Add 250 µL isopropanol to the sample, and vortex for 30 second. Centrifuge briefly to collect any drops from the inside of the lid.

4. Insert a DNA Mini Column into a 2 mL Collection Tube. Transfer the sample from Step 3 to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min at RT.
5. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer DW1 to the DNA Mini Column, invert and mix once, then centrifuge at 10,000 x g for 1 min.

   Note: Buffer DW1 must be diluted with absolute ethanol according to the bottle label before use.
6. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer DW2 to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min.

   Note: Buffer DW2 must be diluted with absolute ethanol according to the bottle label before use.
7. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer DW2 to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min.
8. 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.
9. Transfer the DNA Mini Column into a clean 1.5 mL microcentrifuge tube, add 30-100 µL Buffer AE preheated to 70ºC. Let sit at RT for 5 min, then centrifuge at 10,000 x g for 1 min.

   Note: To improve the yield, repeat this step for a second elution step.
10. Discard the column and store the DNA at -20ºC.

Troubleshooting

Problem	Possible cause and suggestions
Column clogged	• Incomplete Lysis: Extend incubation time with Buffer AL. • Too much sample: Divide sample into multiple tubes and adjust the volume to 250 µL with Buffer AE. • Sample contains solid particles: before adding isopropanol in step 3, centrifuge at 10,000 x g for 3 min to remove undigested impurities, transfer the supernatant to a new centrifuge tube, and then add isopropanol.
Low yield	• Poor Elution: Repeat elution with increased elution volume. Incubate columns at 65°C for 5 minutes with Elution Buffer. • Improper Washing: Buffer DW1, buffer DW2 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: add buffer Buffer DW2 to the column, let sit at RT for 2 min, then centrifuge. • Ethanol contamination: after centrifuging the empty DNA Mini Column at 12,000 x g for 3 min, open cap and let sit at RT for 5-10 min to completely dry the membrane.