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EasySC DNA Purification Kit from animal cells

Catalog Number: D117-1, D117-2

Table 1. Kit Components and Storage

Kit Component	D117-1 (50 preps)	D117-2 (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 animal cells provides rapid total DNA isolation from cultured cells. 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.

Things to do before starting

- Buffer AL may form precipitates upon storage. If necessary, warm to 50°C to redissolve.
- Buffer DW1 and Buffer DW2 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96-100%) as indicated on the bottle to obtain a working solution.
- Preheat a water bath or heating block to 65°C.
- Prepare RNase A solution (10 mg/mL).

Purification Protocol

1. Prepare the cell suspension using one of the following methods:
   - Frozen cell samples should be thawed before starting this protocol. Pellet the cells by centrifugation. Wash the cells with cold PBS (4°C). Resuspend cells in 250 µL PBS. Proceed to Step 2.
   - For cells grown in suspension, pellet 5 x 10^6 by spinning at 1,200 x g in a centrifuge tube. Aspirate and discard the supernatant, and wash the cells once with cold PBS (4°C). Resuspend cells in 250 µL PBS. Proceed to Step 2.

- For cells grown in a monolayer, harvest the cell by either using a trypsin treatment or by scraping with a rubber policeman. Wash cells twice with cold PBS (4°C). Resuspend the cells in 250 µL PBS. Proceed to Step 2.

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 50-100 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. • Insufficient centrifugation: Increase the *g*-force and centrifugation time.
Low yield	• DNA still bound to the membrane: Increase the volume of Buffer AE to 200 µL, and incubate the column at 50°C for 5 min before centrifugation. • Improper Washing: Buffer DW1, buffer DW2 must be diluted with absolute ethanol before use. • Column matrix lost binding capacity during storage: Add 100 µL 3M NaOH to the column prior to loading the sample. Centrifuge at

	10,000 x g for 30 seconds. Add 100 µL water to the columns and centrifuge at 10,000 x g for 30 seconds. Discard the filtrate.
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.