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EasySC DNA Purification Kit from animal tissue

Catalog Number: D113-1, D113-2

Table 1. Kit Components and Storage

Kit Component	D113-1 (50 preps)	D113-2 (200 preps)	Storage	Stability
Buffer ATL	15 mL	60 mL	RT	The product is stable for one year when stored as directed.
Buffer DL	15 mL	60 mL	RT	
Buffer AW1*	15 mL	53 mL	RT	
Buffer AW2*	15 mL	2×25 mL	RT	
Buffer AE	15 mL	30 mL	RT	
Proteinase K (20 mg/mL)	1 mL	4×1 mL	-20℃	
Mini Column	50	200	RT	
2 mL Collection Tube	50	200	RT	

***** Prior to use, add absolute ethanol to **Buffer AW1, Buffer AW2** according to the bottle label.

Product Description

EasySC DNA Purification Kit from animal tissue provides a rapid and easy method for the isolation of DNA from fresh or frozen animal tissue samples. The DNA purification process is simplified with Spin Column technology into four quick “lyse-bind-wash-elute” steps and can be accomplished in less than 20 minutes post-lysis. This convenient spin-column format avoids time-consuming steps like alcohol precipitation, use of toxic compounds such as phenol and chloroform and allows for multiple samples to be processed in parallel. DNA purified using this kit is ready for most downstream applications such as PCR, sequencing, genotyping, southern blot analysis and restriction enzyme digestion.

Features

- ❖ Fast – DNA purification process in less than 20 min post-lysis.
- ❖ Safe – No Phenol/chloroform extractions.
- ❖ High-quality – DNA is suitable for a variety of downstream applications.

Purification Protocol

1. Weigh ~25 mg tissue (or <10 mg liver, lung, or spleen tissue), and cut the tissue into small pieces, then transfer it into a 1.5 mL microcentrifuge tube.

   Note: Using too much tissue sample may reduce yield and purity. Spleen, liver, and kidney tissues are rich in DNA, and should be used no more than 10 mg. Muscle and skin tissues can be used at 30 mg. Liquid nitrogen grinding, mechanical homogenizer, or glass homogenizer can be used to homogenize tissue sample to reduce digestion time.

2. Add 230 µL Buffer ATL and 20 µL Proteinase K solution. Vortex to mix thoroughly.
3. Incubate at 55°C for 1-3 hr or overnight. Vortex briefly every 20-30 min during incubation, or incubate with shaking.

Note: Lysis time depends on the amount and type of tissue used. The average time is 0.5-3 hr, and mouse tails may take 6-8 hr. Lysis can be proceed overnight.

4. (*Optional*) If RNA need be removed, add 20 µL RNase A (10 mg/mL). Mix by invert, and incubate at RT for 15 min.
5. Centrifuge at 12,000 x g for 3 min. Transfer the supernatant to a new 1.5 mL microcentrifuge tube.
6. Add 250 µL Buffer DL. Vortex to mix thoroughly. Incubate at 70°C for 10 min.

   Note: Buffer DL may be precipitated during storage, if happen, heat it at 50^{o}C to dissolve. A precipitate may form upon the addition of Buffer DL. This precipitate does not interfere with the DNA recovery.
7. Add 250 µL 100% ethanol. Vortex to mix thoroughly.

   Note: A precipitate may form upon the addition of ethanol when processing liver or spleen tissue. Pipette up and down 5-10 times to break up the sediment.
8. Connect a DNA Mini Column to a 2 mL Collection Tube. Transfer the entire sample from step 7 into the DNA Mini column including any precipitates that may have formed. Centrifuge at 12,000 x g for 1 min.
9. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW1 to the DNA Mini Column, invert and mix once, then centrifuge at 12,000 x g for 1 min.

   Note: Buffer AW1 must be diluted with absolute ethanol according to the bottle label before use.
10. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW2 to the DNA Mini Column, then centrifuge at 12,000 x g for 1 min.

    Note: Buffer AW2 must be diluted with absolute ethanol according to the bottle label before use.
11. Repeat step 10 for a second wash step.
12. 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.
13. Transfer the DNA Mini Column into a clean 1.5 mL microcentrifuge tube. Add 30-100 µL Buffer AE preheated to 70°C. Close the lid and incubate at RT for 3 min, then centrifuge at 12,000 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
Column clogged	• Incomplete lysis: Extend lysis time with Buffer ATL and Proteinase K solution, or homogenize tissue sample with homogenizer. • Too much sample: Reduce sample amount. Spleen, liver, and kidney tissues are rich in DNA, and should be used no more than 10 mg.
Low yield	• Incomplete homogenization: Completely homogenize sample. • Improper washing: Buffer AW1, buffer AW2 must be diluted with absolute ethanol before use. • Sample has low DNA content: Increase starting material and volume of all reagents proportionally. • Poor elution: Repeat elution with increased elution volume. Incubate columns at 70°C for 5 minutes with Buffer AE.

	• 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: Repeat Buffer AW2 wash twice. • Ethanol contamination: incubate the column at 56°C for 5 min to dry the membrane completely.