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EasySC FFPE DNA Purification Kit

Catalog Number: D125-1, D125-2

Table 1. Kit Components and Storage

Kit Component	D125-1 (50 preps)	D125-2 (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 FFPE DNA Purification Kit is designed for fast and easy purification of DNA from formalin-fixed, paraffin-embedded (FFPE) tissue sections. Paraffin removal can be performed using a xylene and ethanol method. Samples are incubated in a specialized lysis buffer along with Proteinase K to reverse crosslinking, effectively releasing short and long DNA fragments. After adjusting the binding conditions with ethanol, the lysate is applied to the DNA Mini column to bind DNA. Cellular debris and proteins are effectively removed during the wash steps. High quality purified DNA is then eluted in elution buffer and is ready for applications such as PCR and next-generation sequencing.

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.

Things to do before starting

- Buffer ATL, Buffer DL may form precipitates upon storage. If necessary, warm to 50°C to redissolve.
- Buffer AW1 and Buffer AW2 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 56°C and 90°C.
- Prepare RNase A solution (10 mg/mL).

Purification Protocol

1. Use a scalpel to trim excess paraffin off the sample block, and cut the sections into small pieces, then transfer them (<25 mg) into a 2 mL microcentrifuge tube.

   Note: Cutting FFPE tissue section into 10-20 µm slices or small pieces will help to digest.

2. Add 1 mL xylene. Vortex vigorously until paraffin dissolves (e.g., 10-30 s).

3. Centrifuge at 14,000 x g for 2 min at room temperature to collect the tissue pellet. Remove the supernatant by pipetting and discard it. Retain the pellet.

4. Add 1 mL ethanol (96-100%) to the tissue pellet, and mix thoroughly by vortexing.

5. Centrifuge at 14,000 x g for 2 min at room temperature. Remove the supernatant by pipetting and discard it. Retain the pellet.

6. Incubate the open microcentrifuge tube at 37°C for 10-15 min until the ethanol has evaporated.

7. Resuspend the pellet in 230 µL Buffer ATL. Add 20 µL Proteinase K solution, and mix by vortexing.

   Note: Pellet must be well resuspended in the ATL buffer to ensure the maximum yield recovery.

8. Incubate at 56°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. Lysis can be proceed overnight.

9. Incubate at 90°C for 1 hour.

   Note: The incubation at 90°C in Buffer ATL reverses formaldehyde modification of nucleic acids.

10. (*Optional*) If the lysate contains insoluble impurities, centrifuge at 12,000 x g for 3 min. Then, transfer the supernatant to a new 1.5 mL microcentrifuge tube.

11. Add 250 µL Buffer DL to the sample and mix thoroughly by vortexing. Then, add 250 µL ethanol (96-100%) and mix again thoroughly by vortexing.

    Note: Buffer DL may be precipitated during storage, if happen, heat it at 50^{o}C to dissolve. It is essential that the sample, Buffer DL and ethanol are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution. Buffer DL and ethanol can be premixed and added together in one step to save time when processing multiple samples. A precipitate may form upon the addition of Buffer DL and ethanol. This precipitate does not interfere with the DNA recovery.

12. Connect a DNA Mini Column to a 2 mL Collection Tube. Transfer the entire sample from step 11 into the DNA Mini column including any precipitates that may have formed. Centrifuge at 12,000 x g for 1 min.

13. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW1 to the DNA Mini Column, 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.

14. 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.

15. Repeat step 14 for a second wash step.

16. 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.

17. 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.

18. 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. • Too much sample: Reduce sample amount. No more than 25 mg tissue sections. • The lysate contains insoluble impurities: Centrifuge at 12,000 x g for 3 min to remove it.
Low yield	• Insufficient dewaxing: Repeat dewaxing with xylene to completely remove paraffin. • Insufficient decrosslinking: Extend the incubation at 90°C for 90-120 min. • Incomplete lysis: Extend lysis time with Buffer ATL and Proteinase K solution. • Improper washing: Buffer AW1, buffer AW2 must be diluted with absolute ethanol before use. • 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.