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EasyMag FFPE DNA Purification Kit

Catalog Number: D126-1, D126-2

Table 1. Kit Components and Storage

Kit Component	D126-1 (50 preps)	D126-2 (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℃	
MagBinding Beads	2.5 mL	10 mL	2-8 °C	

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

Product Description

EasyMag FFPE DNA Purification Kit is designed for rapid and reliable isolation 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. The released DNA is then purified using magnetic bead technology. Purification requires no phenol or chloroform extraction or alcohol precipitation, and involves minimal handling. Purified DNA is suitable for downstream applications including SNP analysis, sequencing, and genotyping.

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 isopropanol and 50 µL MagBinding Beads, 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 isopropanol are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution. Buffer DL and isopropanol 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 isopropanol. This precipitate does not interfere with the DNA recovery.

12. Incubate with shaking at RT for 5 min.

13. Transfer the tube to a magnetic rack for an additional 2 min or until beads pellet and supernatant is cleared. With the tube on the magnetic rack remove the supernatant and discard.

14. Add 600 µL of Buffer AW1 to the tube. Resuspend the beads by pipetting up and down or vortexing at 1,500 rpm for 30 seconds. Replace the tube on the magnetic rack for 2 min or until beads pellet. Remove and discard the supernatant.

    Note: Buffer AW1 must be diluted with absolute ethanol according to the bottle label before use.

15. Add 600 µL of Buffer AW2 to the tube. Resuspend the beads by pipetting up and down or vortexing at 1,500 rpm for 30 seconds. Replace the tube on the magnetic rack for 2 min or until beads pellet. Remove and discard the supernatant.

    Note: Buffer AW2 must be diluted with absolute ethanol according to the bottle label before use.

16. Repeat step 15 for a second wash step.

17. Leave the tube on the magnetic rack, open the cap and air dry the beads at RT for 10-15 min.

Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.

18. Add 100 µL Buffer AE to the tube, resuspend the beads by vortexing. Incubate at 55ºC for 5 min, vortex for 15 seconds every 1-2 min for 5 min.
19. Replace the tube on the magnetic rack for 2 min or until beads pellet. Transfer the cleared supernatant to a clean tube.
20. Store the DNA at -20ºC.

Troubleshooting

Problem	Possible cause and suggestions
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. • Incomplete resuspension of MagBinding Beads: Resuspend MagBinding Beads by vortexing virgously before use. • DNA remains bound to MagBinding Beads: Increase elution volume and incubation time. • Loss of MagBinding Beads during operation: Avoid disturbing MagBinding Beads during aspiration.
Poor performance in downstream applications	• Salt contamination: Remove any residual liquid with a pipettor after each wash step. • Ethanol contamination: Ensure the beads are completely dried before elution.