Ver. 23111

ODP317

FFPE DNA Kit

For DNA purification from formalin fixed, paraffin embedded tissues

Origin®

ISO 13485:2016 ISO 9001:2015

FFPE DNA Kit

Spin Column
(ODP317)

Kit Contents

Contents	(50 preps)
Buffer AR (10X)	1.5mL
Buffer GA	10mL
Buffer GB	10mL
Buffer GD	13mL
Buffer PW	15mL
Buffer TE	10mL
Proteinase K (20mg/mL)	1mL
Spin column CB3	50
Collection Tubes (2mL)	50
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Storage

FFPE DNA kit could be stored dry at room temperature (15-25°C) for up to 12 months without showing any reduction in performance and quality. For longer term storage, the kit could be stored at 2-8°C.

(Note: Check buffers for precipitate before use and dissolve at 37°C for 10minutes if necessary)

Proteinase K should be stored at -20°C

Introduction

FFPE DNA Kit is optimized for purification of DNA from formalin fixed and paraffin embedded tissue sections. It uses xylene to remove paraffin, and provides unique lysis conditions for DNA release from tissue slice, well removes formalin crosslinking of the released DNA. Combined selective-binding silica-based membrane and flexible elution system, this kit could elute high-quality DNA.

DNA purified by FFPE DNA Kit is stable and of high purity and is suited for PCR and Real-time PCR analysis, SNP genotype analysis and STR analysis, and pharmacogenomics analysis.

Important Notes

1. Fix tissue samples in 4-10% formalin for 8-24 hours immediately after surgical removal. Longer fixation time will lead to severe DNA fragmentation, resulting in poor performance in downstream assays.
2. Thoroughly dehydrate samples prior to embedding (residual formalin can inhibit PCR reaction).
3. The integrity of DNA obtained with this kit depends on the samples type, storage and fixation conditions. Longer fixation time or longer storage time (>1 year) will lead to DNA fragmentation and in this case, long fragments will not be amplified.
4. Add appropriate amount of ethanol (96-100%) to Buffer GD and Buffer PW as indicated on the bottle before use.
5. All centrifugation steps are carried out in conventional tabletop microcentrifuge at room temperature.
6. Increasing the time of absorption and elution could improve recovery efficiency.
7. The recovery efficiency is related to starting DNA quantity and elution volume.
8. If a precipitate has formed in Buffer GB or Buffer GD, warm buffer to 56°C until the precipitate has fully dissolved.
9. Repeated freezing and thawing of Proteinase K should be avoided; otherwise it would reduce the DNA quality and quantity.

Protocol

Ensure that Buffer GD and Buffer PW have been prepared with appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly.

1. Preparation of samples
   a) Paraffin section: 5-8 pieces of paraffin sections (thickness of 5-10 μm, surface area of 1×1 cm^2).
   b) Paraffin block: Use a scalpel to cut around 30mg tissue sample (trim excess paraffin off).
   Note: If sample surface has been exposed to air, discard the first 2-3 sections.

- Place the paraffin section or paraffin block in a 1.5 mL centrifuge tube, and add 1 mL xylene to the sample. Close the lid and vortex vigorously for 10 seconds.
- Centrifuge at 12,000 rpm (~13,400 × g) for 2 minutes at room temperature. Remove the supernatant by pipetting.
- **Note: Do not remove any pellet.**
- Add 1ml ethanol (96-100%) to the pellet and vortex for 10 seconds.
- Centrifuge at 12,000 rpm (~13,400 × g) for 2 minutes at room temperature. Remove the supernatant by pipetting.
- **Note: Do not remove any pellet.**
- Keep the tube opened at room temperature for 5-10 minutes or until all the residual ethanol has evaporated.

c) Formalin fixed tissue: Use a scalpel to cut around 30mg tissue sample into small pieces and place in a 1.5mL microcentrifuge tube. Add 500µL PBS (10mM; pH 7.4) mix by vortexing and centrifuge for 1 minute at 12,000 rpm (~13,400 ×g), discard the supernatant and wash the pellet with 500µL PBS.

- Add 200µL NaCl (3M; pH 7.0) mix by vortexing and centrifuge for 1 minute at 12,000 rpm (~13,400 ×g), discard the supernatant and wash the pellet with 200µL NaCl.
- Add 200µL Buffer AR (1X) and mix by vortexing and incubate at 100°C for 20 minutes and centrifuge for 1 minute at 12,000 rpm (~13,400 ×g).

  Note: From the concentrated 10X solution, 1X working solution can then be prepared as needed by further dilution of the 10X stock in dH_2O. The pH of 1X Buffer should be brought to 6.0 before making up the volume to 1X concentration. Do not store the diluted 1X buffer for future use.

2. Re-suspend the pellet in 200µL Buffer GA.
3. Add 20µL Proteinase K, mix thoroughly by vortexing. at 56°C until the tissue is completely lysed.

   Optional Step: Add 2µL RNase A (10mg/mL) (Cat# ORT405) to remove RNA.
4. Add 200µL Buffer GB to the sample, mix thoroughly by vortexing, and incubate at 70°C for 10 minutes to yield a homogeneous solution. Briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from the inside of the lid.

   Note: Precipitates are expected, but it will not interfere with the extraction.
5. Add 200µL ethanol (96-100%) to the sample, and mix thoroughly by vortexing for 20 seconds. Briefly centrifuge the 1.5mL microcentrifuge tube to remove drops from the inside of the lid.

   Note: Precipitate formed in the earlier step will be dissolved.
6. Pipet the mixture from previous step into the spin column CB3 (in a 2mL collection tube) and centrifuge at 12,000 rpm (~13,400 ×g) for 45 seconds. Discard flow-through and place the spin column into the collection tube.
7. Add 500µL Buffer GD to spin column CB3, and centrifuge at 12,000rpm (~13,400 ×g) for 30 seconds, then discard the flow-through and place the spin column into the collection tube.
8. Add 700µL Buffer PW to spin column CB3, and centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds. Discard the flow-through and place the spin column into the collection tube.
9. Repeat step 8.
10. Centrifuge at 12,000 rpm (~13,400 ×g) for 2 minutes to dry the membrane completely.

    Note: The resident ethanol of buffer PW may have some affect in downstream application.
11. Place the spin column CB3 in a new clean 1.5 mL microcentrifuge tube, and pipette 50-200µL Buffer TE or distilled water directly to the center of the membrane. Incubate at room temperature (15–25°C) for 2-5 minutes, and then centrifuge for 2 minutes at 12,000 rpm (~13,400 × g).

Note: To increase the DNA yield, introduce the eluted Buffer TE to the column and centrifuge for 2 minutes at 12,000 rpm.

If the volume of eluted buffer is less than 50µL, it may affect recovery efficiency. The pH of eluted buffer will influence the elution, we suggest chose buffer TE or distilled water (pH 7.0 - 8.5) to elute DNA. For long-term storage of DNA, eluting in Buffer TE and storing at –20°C is recommended, since DNA stored in water is subject to acid hydrolysis.