FastPure FFPE DNA Isolation Kit

Catalog# DC105

Version 8.1

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01/ Introduction

FastPure FFPE DNA Isolation Kit is suitable for the separation and purification of genomic DNA from formalin-fixed paraffin-embedded (FFPE) tissues, overcoming the inhibitory effects caused by formalin crosslinking of nucleic acids. The Deparaffinization Solution used in the kit is safer than xylene; High-quality DNA can be obtained by a centrifugal adsorption column that specifically binds DNA and a unique buffer system. The whole extraction process takes only 20 min (except digestion time). The extracted genome with good integrity, high purity, stable and reliable quality can be used in various downstream applications including PCR, qPCR, library construction.

02/ Components

Components	DC105 (50 rxn)
Deparaffinization Solution	40 ml
Buffer FTL	15 ml
Buffer FL	15 ml
Buffer FW1	13 ml
Buffer FW2	20 ml
Proteinase K	22 mg
Proteinase K Dissolve Solution	2 ml
Elution Buffer	10 ml
FFPE DNA Mini Columns	50 Columns
2 ml Collection Tubes	50 Columns

Deparaffinization Solution: for the removal of paraffin in samples.

Buffer FTL: sample suspension solution and lysis buffer.

Buffer FL: providing binding condition of the DNA to the membrane.

Buffer FW1: for the removal of protein residues.

Buffer FW2: for the removal of salt ions residues.

Proteinase K: for enzyme digestion of tissue samples.

Proteinase K Dissolve Solution: for dissolving Proteinase K powder.

Elution Buffer: for the elution of genomic DNA.

FFPE DNA Mini Columns: for specifically adsorbing genomic DNA.

2 ml Collection Tubes: collection tube for filtrate.

03/ Storage

All components (except Proteinase K) are stored at 15°C - 25°C for 12 months. For long-term storage, please place at 2°C - 8°C. The buffer may precipitate when stored at low temperature. Dissolve at room temperature for a while, if necessary, or preheat at 37°C for 10 min to thaw the precipitation and mix thoroughly before use.

04/ Additional Materials Required

1.56°C and 90°C water bath;

2.Absolute ethanol;

3.Prepare Proteinase K working solution: add 1.1 ml of Proteinase K Dissolve Solution to 22 mg Proteinase K to a final concentration of 20 mg/ml, and gently invert to dissolve Proteinase K. Proteinase K powder can be stored at 2°C - 8°C for one year, but the dissolved Proteinase K working solution must be stored at -20°C, avoid repeated freezing and thawing.

4.Buffer FW1 should be diluted with 17 ml of absolute ethanol according to indication on the bottle label before use.

5.Buffer FW2 should be diluted with 80 ml of absolute ethanol according to indication on the bottle label before use.

05/ Tips

◇ Fresh sections of tissue samples should be fixed in 4 - 10% formalin as quickly as possible, and the fixation time should be within 8 - 24 h. Over-long fixation time leads to DNA fragmentation, resulting in poor performance in downstream extraction assays.

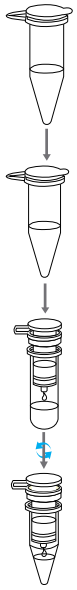
◇Thoroughly dehydrate samples prior to embedding (residual formalin can inhibit the subsequent PCR detection).

◇The integrity of the extracted DNA depends on the sample types, storage time, and fixation conditions. For samples with over-long fixation time by formalin or over-long storage time (>1 year), DNA integrity is impaired and long fragments cannot be extended.

◇If a precipitate appears in Buffer FTL or Buffer FL, it is recommended to dissolve at room temperature for a while or at 37°C water bath for 10 min to thaw the precipitation and mix thoroughly before use.

◇Each reagent in the kit should be operated in accordance with the instructions before use.

06/ Principle and Procedure



★ Take 5 - 8 paraffin sections (total weight <20 mg, each section with thickness of 5 - 10 μm and surface area of 1 × 1 cm^2);
Add 0.6 ml of Deparaffinization Solution;
Incubate in 56°C water bath for 6 min;
Centrifuge at 14,000 × g for 2 min and discard the supernatant.

★ Add 200 μl of Buffer FTL and 20 μl of Proteinase K working solution;
Incubate in 56°C water bath for 1 h (until the sample is completely lysed);
Incubate in 90°C water bath for 1 h (removing the crosslinking of protein and DNA);
Add 200 μl of Buffer FL and 200 μl of absolute ethanol.

★ Transfer the mixture to the adsorption column, centrifuge at 10,000 × g for 30 - 60 sec, and discard the filtrate;
Add 500 μl of Buffer FW1 (with ethanol) to the adsorption column, centrifuge at 10,000 × g for 30 - 60 sec, and discard the filtrate;
Add 650 μl of Buffer FW2 (with ethanol) to the adsorption column, centrifuge at 10,000 × g for 30 - 60 sec, and discard the filtrate;
Centrifuge empty adsorption column at 10,000 × g for 3 min.

★ Add 15 μl- 50 μl of Elution Buffer. Incubate at room temperature for 1 min, centrifuge at 10,000 × g for 1 min, and collect the filtrate.

07/ Protocol

1.Please add the corresponding volume of absolute ethanol to Buffer FW1 and Buffer FW2 according to indication on the bottle label before use.

2.Prepare Proteinase K working solution using Proteinase K Dissolve Solution to dilute Proteinase K into a final concentration of 20 mg/ml.

Extraction Steps

1.Take 5-8 paraffin sections (each section with thickness of 5 - 10 μm and surface area of 1 × 1 cm^2), remove excess paraffin off the sample by a scalpel, cut up the sample (<20 mg) into as small pieces as possible, and transfer into a 1.5 ml centrifuge tube.

▲Removing excess paraffin or cutting up the sample into small pieces with scissors and scalpels facilitates subsequent deparaffinization.

2.Add 0.6 ml of Deparaffinization Solution to the sample, vortex vigorously for 5 sec. Briefly centrifuge the tube to make the sample immerse in Deparaffinization Solution, and incubated at 56 ° C for 6 min, then vortex vigorously for 20 sec.

3.Centrifuge at 14,000 × g for 2 min and discard the supernatant. Note: Do not remove any of the precipitate.

4.Add 200 μl of Buffer FTL and 20 μl of Proteinase K working solution to the sample and mix by vortexing. Incubate in 56°C water bath for 1 h (until the sample is completely lysed), mix the tube by inverting for several times during incubation.

5.Incubate in 90°C water bath for 1 h. Note: Treatment at 90°C can remove the crosslinking of DNA and protein, significantly increasing DNA yield.

▲(Optional) If undigested impurities are still present in the digestion buffer, centrifuge at 10,000 × g for 3 min to remove the impurities. Transfer the supernatant to a new centrifuge tube.

07/ Protocol

Extraction Steps

6.Add 200 µl of Buffer FL and 200 µl of absolute ethanol to the above treatment solution and vortex for 15 sec.

7.Place the FFPE DNA adsorption column in a collection tube and transfer the mixture to the adsorption column. Centrifuge at 10,000 × g for 60 sec.

8.Discard the filtrate and reuse the collection tube. Add 500 µl of Buffer FW1 (with ethanol) to the adsorption column, and centrifuged at 10,000 × g for 60 sec.

9.Discard the filtrate and reuse the collection tube. Add 650 µl of Buffer FW2 (with ethanol) to the adsorption column, and centrifuged at 10,000 × g for 60 sec.

10.Discard the filtrate, centrifuge empty adsorption column at 10,000 × g for 3 min to dry the membrane completely.

11.Transfer the adsorption column to a new 1.5 ml centrifuge tube, open the lid for 3 min to volatilize ethanol completely. Add 15 µl - 50 µl of Elution Buffer to the center of the adsorption column. Incubate at room temperature for 1 min, and centrifuge at 10,000 × g for 1 min.

12.Discard the adsorption column, store the eluted DNA at -20 °C, or store at -70 °C for long-term storage.

Troubleshooting

Problem	Possible Reason	Recommended Solutions
Column is clogged.	1. Excessive sample input.	Reduce the amount of sample, do not exceed 8 to 10 of paraffin sections.
	2. Insufficient removing paraffin.	Scrap off excess paraffin while processing the sample. Increase the amount of Deparaffinization Solution or perform deparaffinization step again to completely remove paraffin.
	3. Insufficient sample lysis.	Increase the amount of Proteinase K working solution or extend the lysis time of Proteinase K working solution.
	4. Undigested impurities are present in the digestion buffer.	Centrifuge at 10,000 × g for 3 min to remove the impurities.
Low DNA yield.	1. Low DNA content in sample.	Use a sample with a short storage time or a fresh sample for extraction; Appropriately increase the amount of tissue samples.
	2. Insufficient sample lysis.	Increase the amount of Proteinase K working solution or extend the lysis time of Proteinase K working solution.
	3. Absolute ethanol is not added to Buffer FW1 or Buffer FW2.	Add the correct volume of absolute ethanol according to the label instructions.
	4.Over-drying of the adsorption column before adding the Elution Buffer.	The opening time of the adsorption column lid should not exceed 5 min, otherwise it will cause low efficiency of DNA elution.
	5.Insufficient elution.	The Elution Buffer should be added to the center of the adsorption film. Increase the elution volume and the number of elution or extend elution time.
Low DNA purity.	1.Protein residue.	Ensure that there is no precipitation in the sample solution when adding Buffer FL and absolute ethanol to the sample; Centrifuge at correct speed when purifying the adsorption column with Buffer FW1.
	2. Salt ions residue.	Wash the column by Buffer FW2 twice.
	3. Ethanol residue.	Ensure that the empty tube is centrifuged and the lid is opened at room temperature to volatilize ethanol completely.
RNA contamination.	RNA is extracted during sample extraction.	Equilibrate the sample to room temperature after removing the crosslinking at 90°C, and add 2 µl of RNase A (100 mg/ml) to the sample, then incubated for 2 min at room temperature.

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