FastPure FFPE DNA Isolation Kit

DC105

Instruction for Use

Version 23.1

Vazyme

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01/Product Description

This kit is intended for isolating genomic DNA from formalin-fixed, paraffin-embedded (FFPE) tissue, overcoming the inhibitory effect caused by formalin crosslinking. The deparaffinization solution in this kit is safer than xylene. The DNA-specific spin column and the unique buffer system also ensure the high quality of obtained DNA. The whole extraction process takes only 20 min (digestion time not counted). The extracted genomic DNA is of good integrity, high purity, and stable quality and can be used for a wide range of downstream applications, including PCR, qPCR, and library preparation.

02/Components

Components	DC105-01 (50 rxns)
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
2 ml Collection Tubes	50

Deparaffinization Solution: Remove the paraffin in the sample.
Buffer FTL: Suspend the sample and lyses tissue cells.
Buffer FL: Provide an environment for DNA binding.
Buffer FW1: Remove proteins from DNA.
Buffer FW2: Remove salt ions from DNA.
Proteinase K: Lyse tissue samples.
Proteinase K Dissolve Solution: Dissolve the Proteinase K powder.
Elution Buffer: Elute DNA from the spin column.
FFPE DNA Mini Columns: Specifically adsorb genomic DNA in samples.
2 ml Collection Tubes: Collect filtrate.

03/Storage

Proteinase K powder: Store at 2 ~ 8°C and adjust the shipping method according to the destination.
Store dissolved Proteinase K at -30 ~ -15°C.
Store the other components at 15 ~ 25°C and transport at room temperature.

04/Applications

Paraffin-embedded tissue sections: 5 - 8 sections (5 - 10 μm thick, 1 × 1 cm^2 in size, <20 mg).

05/Self-prepared Materials

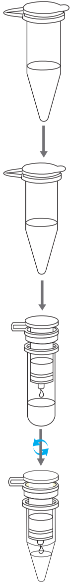
1. 56°C and 90°C water bath.
2. Absolute ethanol.
3. Prepare Proteinase K working solution: Add 1.1 ml of Proteinase K Dissolve Solution to 22 mg of Proteinase K to a final concentration of 20 mg/ml. Gently invert the container until complete dissolution. Proteinase K powder can be stored at 2 ~ 8°C for one year, but the reconstituted working solution should be stored at -30 ~ -15°C in aliquots, as repeated freezing and thawing will compromise its activity.
4. Add 17 ml of absolute ethanol to Buffer FW1 before use according to the label.
5. Add 80 ml of absolute ethanol to Buffer FW2 before use according to the label.

06/Notes

For research use only. Not for use in diagnostic procedures.

1. Fresh tissue sections should be fixed with 4% - 10% formalin as soon as possible for 8 - 24 h. Too long formalin fixation may result in fragmented genomic DNA and hinder further extraction.
2. Ensure that samples are thoroughly dehydrated before embedding, as residual formalin can adversely affect the detection by PCR.
3. The integrity of the DNA extracted with this kit depends on the sample type, storage time, and fixation conditions. If the sample is fixed with formalin or stored for too long (>1 year), the DNA integrity may be compromised, and the amplification of long fragments may not be accomplished.
4. If any precipitate is observed in Buffer FTL or Buffer FL, keep the buffer at room temperature or incubate it in a water bath at 37°C for 10 min until the precipitate is dissolved.
5. Prepare the reagents in the kit before use as per the Instructions for Use.

07/Mechanism & Workflow



★ Take 5 - 8 paraffin sections with a thickness of 5 - 10 μm and a size of 1 × 1 cm^2 (<20 mg).
Add 0.6 ml of Deparaffinization Solution.
Incubate in a 56°C water bath for 6 min.
Centrifuge at 10,200 rpm (10,000 × g) for 2 min and discard the supernatant.

★ Add 200 μl of Buffer FTL and 20 μl of Proteinase K working solution.
Incubate in a 56°C water bath for 1 h (until complete sample digestion).
Incubate in a 90°C water bath for 1 h (to reverse DNA-protein crosslinking).
Add 200 μl of Buffer FL and 200 μl of absolute ethanol.

★ Transfer the mixture to a spin column, centrifuge at 10,200 rpm (10,000 × g) for 1 min, and discard the filtrate.
Add 500 μl of Buffer FW1 (supplemented with ethanol) to the spin column, centrifuge at 10,200 rpm (10,000 × g) for 1 min, and discard the filtrate.
Add 650 μl of Buffer FW2 (supplemented with ethanol) to the spin column, centrifuge at 10,200 rpm (10,000 × g) for 1 min, and discard the filtrate.
Centrifuge empty column at 10,200 rpm (10,000 × g) for 3 min.

★ Add 15 - 50 μl of Elution Buffer. Leave at room temperature for 1 min, centrifuge at 10,200 rpm (10,000 × g) for 1 min, then collect the filtrate.

08/Experiment Process

1. Before use, add the specified volumes of absolute ethanol to Buffer FW1 and Buffer FW2 according to the label.
2. Prepare Proteinase K into a working solution (20 mg/ml final concentration) with Proteinase K Dissolve Solution.

Extraction Steps

1. Take 5 - 8 paraffin sections (5 - 10 μm thick, 1 × 1 cm^2 in size). Remove excess paraffin with a clean razor, and cut the sample (<20 mg) into pieces as small as possible. Transfer the pieces into a 1.5 ml centrifuge tube.

   ▲ This step facilitates subsequent deparaffinization.
2. Add 0.6 ml of Deparaffinization Solution to the sample and vortex vigorously for 5 sec. Briefly centrifuge to immerse the sample in the Deparaffinization Solution. Incubate the sample in a 56°C water bath for 6 min, and vortex vigorously for 20 sec.
3. Centrifuge at 10,200 rpm (10,000 × g) for 2 min and discard the supernatant. Note: Do not disturb the precipitation.
4. Add 200 μl of Buffer FTL and 20 μl of Proteinase K working solution to the sample, and mix well by vortexing. Incubate the sample in a 56°C water bath for 1 h until complete digestion. Mix the sample by inversion a few times during the incubation.

5. Incubate in a 90°C water bath for 1 h. Note: Heating at 90°C reverses DNA-protein crosslinking, significantly enhancing DNA yield.
   ▲ (Optional) If the lysate still contains undigested impurities, centrifuge at 10,200 rpm (10,000 × g) for 3 min to remove these impurities. Transfer the supernatant to a new centrifuge tube.
6. Add 200 μl of Buffer FL and 200 μl of absolute ethanol to the above mixture and vortex for 15 sec to mix well.
7. Place FFPE DNA Mini Columns in a 2 ml Collection Tubes, and transfer the mixture to the spin column. Centrifuge at 10,200 rpm (10,000 × g) for 1 min.
8. Discard the filtrate and place the FFPE DNA Mini Columns in the 2 ml Collection Tubes. Add 500 μl of Buffer FW1 (supplemented with ethanol) to the spin column and centrifuge at 10,200 rpm (10,000 × g) for 1 min.
9. Discard the filtrate and place the FFPE DNA Mini Columns in the 2 ml Collection Tubes. Add 650 μl of Buffer FW2 (supplemented with ethanol) to the spin column and centrifuge at 10,200 rpm (10,000 × g) for 1 min.
10. Discard the filtrate and place the FFPE DNA Mini Columns in the 2 ml Collection Tubes. Centrifuge the empty column at 10,200 rpm (10,000 × g) for 3 min.
11. Transfer the FFPE DNA Mini Columns into a new 1.5 ml centrifuge tube, and leave the tube uncapped for 3 min to allow the ethanol to evaporate. Add 15 - 50 μl of Elution Buffer to the center of the membrane. Incubate at room temperature for 1 min, and centrifuge at 10,200 rpm (10,000 × g) for 1 min.
12. Discard the FFPE DNA Mini Columns and store the DNA at -20°C. For long-term storage, keep at -70°C.

09/FAQ & Troubleshooting

FAQ	Reasons	Solutions
Clogging of the spin column	1. Too much sample	Reduce the sample amount. Do not use more than 8 - 10 paraffin sections.
	2. Incomplete deparaffinization	Scrape off excess paraffin when processing the sample. Increase the volume of the deparaffinization solution or perform a second deparaffinization step to remove paraffin completely.
	3. Incomplete sample digestion	Increase the volume of the Proteinase K working solution or prolong the digestion.
	4. Insoluble substances in the lysate	Centrifuge at 10,200 rpm (10,000 × g) for 3 min to remove the undigested substances.
Low DNA yield	1. Low DNA content in the sample	Use a sample that has not been stored for a long time or a fresh sample for DNA extraction, or increase the amount of tissue sample as appropriate.
	2. Incomplete sample digestion	Increase the volume of Proteinase K working solution or prolong the digestion to completely lyse the sample.
	3. Buffer FW1/FW2 not supplemented with absolute ethanol	Add appropriate volumes of absolute ethanol according to the label.
	4. Spin column overdried before addition of Elution Buffer	Do not air dry the spin column for over 5 min, or DNA may become difficult to elute.
	5. Incomplete elution	Add Elution Buffer to the center of the spin column membrane and increase the volume and number of elutions.
Low DNA purity	1. Residual proteins	Make sure that there is no precipitate in the sample before adding Buffer FL and absolute ethanol. Use the correct centrifugation speed when washing the spin column with Buffer FW1.
	2. Residual salt ions	Wash the spin column twice with Buffer FW2.
	3. Residual ethanol	Make sure that the spin column is air dried at room temperature after the last centrifugation to allow for the complete evaporation of ethanol.
RNA contamination	1. RNA is extracted	After reverse crosslinking at 90°C, add 2 µl of RNase A (100 mg/ml) to the sample after it returns to room temperature, and incubate for 2 min.

Vazyme

Nanjing Vazyme Biotech Co.,Ltd.

Tel: +86 25-83772625

Email: info.biotech@vazyme.com

Web: www.vazyme.com

Loc: Red Maple Hi-tech Industry Park, Nanjing, PRC

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www.vazyme.com