ALEXGEN gDNA EXTRACTION KIT FROM FFPE TISSUE

FOR EXTRACTION OF GDNA FROM FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUE SAMPLES.

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Alexius Biosciences

Decoding Life

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KIT CONTENTS

Proteinase K solution	**1 mL**
FFPE tissue lysis buffer	15 mL
Binding buffer※	15 mL
WB I (concentrate)※	12 mL
WB II (concentrate)	8 mL
Elution buffer	10 mL
DNA spin column with collection tube	50 Nos.

※**Binding buffer** and **WB I** contains salts of hydrochloride which is not compatible with bleach containing disinfecting agents. In case of spill, remove buffer using water first, then disinfect that place using disinfecting reagent.

USER SUPPLIED EQUIPMENT AND REAGENTS

- Centrifuge, centrifuge rotor for 2 mL tube.
- Xylene for removal of paraffin.
- Ethanol (96- 100%).
- 1.5 mL microfuge tubes, 2 mL microfuge tubes.
- Water bath/ heating block.
- RNase A (in case of RNA free gDNA is required)
- Nuclease free distilled water.

NOTES BEFORE STARTING

PREPARATION AND STORAGE OF REAGENTS

PROTEINASE K

Proteinase K solution supplied with this kit must be stored at -20° C. Proteinase K solution stored at -20° C is stable for 12 months. Before each use, make sure that solution is properly thawed.

LYSIS BUFFER

Before starting, mix lysis buffer well. If precipitates are formed during storage, it can be dissolved by incubating buffer at 55° C.
Note: Do not add Proteinase K directly to lysis buffer.

WB I

WB I provided with kit is in form of concentrate. Add **16 mL** 96- 100% ethanol to it before first use. Close the lid of bottle properly after each use.
WB I is stable for 6 months after addition of ethanol.

WB II

WB II provided with kit is in form of concentrate. Add **20 mL** 96- 100% ethanol to it before first use. Close the lid of bottle properly after each use.
WB II is stable for 6 months after addition of ethanol.

ELUTION BUFFER

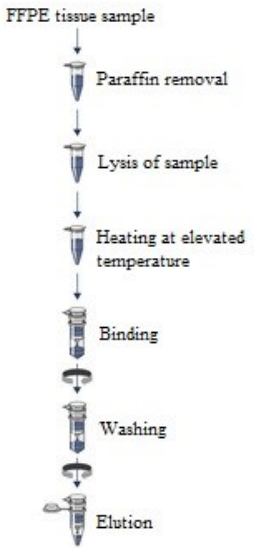
Elution buffer can be stored at room temperature. Pre-warm elution buffer at 70° C every time before use.

SAMPLE PREPARATION

Sample should be freshly cut section of FFPE tissue block. Cut sections in size of 5-10 µm and try to avoid paraffin as much as possible. In single reaction four to five sections can be used for the isolation. Adding more than required sample to the reaction will lead to improper digestion of tissue and low yield of DNA at the end of the process.

PRINCIPLE

This kit works on solid phase extraction method to quickly purify DNA. There are mainly six steps are involved in this method, 1-Removal of paraffin, 2- Cell lysis, 3- Heating of sample at elevated temperature, 4- Binding of DNA to spin column, 5- Washing/ removal of contaminants and 6- Final elution of purified DNA.



Understanding the solid phase extraction of DNA.

First step is to remove the paraffin. Paraffin removal is necessary as it blocks the digestion of the sample during lysis process. After removal, sample is subjected to lysis process. During the lysis step lysis buffer destabilizes proteins of the cell including cellular enzymes which restricts the nuclease activity. After lysis is completed, sample is incubated at elevated temperature, generally at 90°C which will partially reverse the effect of formaldehyde modification of nucleic acid. After incubation is completed, sample is subjected to binding. Binding buffer will disrupt the nucleic acid from water which is required for nucleic acid binding to spin column membrane. Addition of ethanol during binding process is for enhancing the nucleic acid binding process. Two wash steps are for the removal for the protein and salts bounded to the spin column membrane. Ethanol will interfere with downstream process such as PCR, to remove ethanol present in DNA spin column, there is a drying step involved in this process. At the end we are adding elution buffer to separate the DNA from the spin column.

PROCEDURE

BEFORE YOU START

- Make sure you have added ethanol to WB I and WB II before first use.
- Heat water bath/ heating block to 55°C for use at step 5.
- If precipitate has formed in tissue lysis buffer and binding buffer then dissolve by incubating at 55°C.
- Use pre warmed elution buffer (65°- 70°C) at step 13.
- All centrifugation steps performed in this protocol are at 15°- 20°C.

PROTOCOL FOR GDNA EXTRACTION FROM FFPE TISSUE SAMPLES

1. PREPARE SAMPLE BY CUTTING SECTIONS OF 5-10µM. PREPARE UP TO 5 SECTIONS AND REMOVE AS MUCH AS POSSIBLE PARAFFIN USING SCALPEL.
2. TRANSFER SECTIONS TO STERILE 1.5ML VIAL AND ADD 1ML XYLENE TO IT AND CLOSE THE LID. VORTEX VIGOROUSLY FOR AT LEAST 10 SECONDS.
3. CENTRIFUGE THE TUBE AT FULL SPEED FOR 2 MINUTES AND REMOVE SUPERNATANT AND ADD 1ML 96-99% ETHANOL TO THE PELLET. MIX BY VORTEXING FOR AT LEAST 10 SECONDS.
   Ethanol addition is required to remove residual Xylene from the sample which may cause problem in later steps.
4. CENTRIFUGE AT FULL SPEED FOR 2 MINUTES AND REMOVE SUPERNATANT WITHOUT DISTURBING PELLET. TRY TO REMOVE ETHANOL AS MUCH AS POSSIBLE USING PIPETTE. OPEN THE LID OF VIAL AND LET THE ETHANOL EVAPORATE.
   To remove ethanol efficiently, you can heat sample at 37°C with open lid for 10 minutes. Temperature above 37°C is not recommended.
5. ADD 180ML TISSUE LYSIS BUFFER AND 20ML PROTEINASE K SOLUTION TO THE SAMPLE PELLET. MIX BY VORTEXING THE TUBE FOR 10 SECONDS AND INCUBATE AT 55°C FOR ONE HOUR OR UNTIL SAMPLE HAS BEEN COMPLETELY LYSED.
6. INCUBATE SAMPLE AT 90°C FOR ONE HOUR.
   The incubation at 90°C for one hour helps to partially reverses formaldehyde modification of nucleic acids. Longer incubation period or higher incubation temperature will result in more fragmented DNA. If using only one heating block, leave the sample at room temperature after the 55°C incubation until the heating block has reached 90°C.

7. BRIEFLY CENTRIFUGE TO REMOVE DROPS FROM THE INSIDE OF LID. ADD 200ML BINDING BUFFER AND MIX BY PIPETTING FOR AT LEAST SIX TIMES. INCUBATE AT 55°C FOR 10 MINUTES.

8. BRIEFLY CENTRIFUGE TO REMOVE DROPS FROM THE INSIDE OF LID. ADD 200ML 96-99% CHILLED ETHANOL TO THE SAMPLE AND MIX BY INVERTING 8-10 TIMES (DO NOT VORTEX). AFTER MIXING, BRIEFLY CENTRIFUGE TO REMOVE DROPLETS FROM THE INSIDE OF THE LID.

9. PLACE A SPIN COLUMN ON COLLECTION TUBE PROVIDED IN KIT AND TRANSFER THE CONTENT FROM STEP 8 TO THE COLUMN. CENTRIFUGE AT 6000X G FOR 1 MINUTE. DISCARD THE FILTRATE AND REPLACE THE COLUMN ON COLLECTION TUBE.
   Spin column can hold 600µL volume at a time. If the sample volume is larger than 600µL, than user need to perform step 9 multiple times.
   After performing step 9, make sure that the all sample has passed through the column and column is not clogged. If the sample has not completely passed through the column, centrifugation at 10,000X g is recommended.

10. ADD 500ML WB1 TO SPIN COLUMN AND CENTRIFUGE AT 6000X G FOR 1 MINUTE. DISCARD THE FILTRATE AND REPLACE THE COLUMN ON COLLECTION TUBE.

11. ADD 500ML WB2 TO SPIN COLUMN AND CENTRIFUGE AT 6000X G FOR 1MINUTE. DISCARD THE FILTRATE AND PLACE THE SPIN COLUMN ON FRESH 2ML MICROCENTRIFUGE TUBE (USER SUPPLIED).

12. CENTRIFUGE AT 20,000X G FOR 3 MINUTES. DISCARD 2ML MICROCENTRIFUGE TUBE.
   This step helps to eliminate the chance of possible ethanol carryover.

13. PLACE THE COLUMN ON CLEAN FRESH 1.5/ 2ML TUBE (USER SUPPLIED). ADD 50ML PRE WARMED (65°- 70°C) ELUTION BUFFER TO THE CENTER OF THE SPIN COLUMN MEMBRANE. INCUBATE COLUMN AT ROOM TEMPERATURE FOR 1 MINUTE.

14. CENTRIFUGE AT 20,000X G FOR 1 MINUTE.

15. REPEAT STEP 13 AND 14 TO INCREASE YIELD BY UP TO 15%. DISCARD COLUMN AND STORE ELUTED DNA AT -20°C OR -80°C.

TROUBLESHOOTING GUIDE

Problem	Probable cause	Solution
Clogged spin column	Inefficient cell lysis due to insufficient addition of Lysis buffer/ improper lysis condition.	Add sufficient amount of lysis buffer as mentioned in protocol. Incubate at recommended temperature for recommended time.
	Inefficient lysis due to improper paraffin removal	Remove paraffin before properly before proceeding to lysis step
Low or no DNA yield	Inefficient cell lysis due to insufficient addition of Lysis buffer/ improper lysis condition.	Add sufficient amount of lysis buffer as mentioned in protocol. Incubate at recommended temperature for recommended time.
	Improper washing	Make sure you have added ethanol to WB I and WB II as mentioned in Preparation and storage of reagents section. Make sure to Close the lid of bottle properly after each use.
Low A_{260}/A_{280} ratio of eluted DNA	No ethanol added to the sample before loading to the spin column	Repeat the extraction procedure with new sample.
	Improper incubation at elution step	Repeat elution step carefully
	Elution with higher amount of elution buffer than the recommended volume	Use recommended volume of elution buffer
	Inefficient cell lysis due to insufficient addition of Lysis buffer/ improper lysis condition.	Add sufficient amount of lysis buffer as mentioned in protocol. Incubate at recommended temperature for recommended time.
	Improper washing	Make sure you have added ethanol to WB I and WB II as mentioned in Preparation and storage of reagents section. Make sure to Close the lid of bottle properly after each use.