RUO

REF	G2MBR4-0652	50 Tests
	G2MBR4-0653	250 Tests

SPINXT FFPE DNA Extraction Kit

Intended Use

The SpiNXT FFPE DNA Extraction Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of neither a disease nor it is suitable for administration to humans or animals. All due care and attention should be exercised in the handling of the products.

Intended User

The assay is intended to be performed by a laboratory professional in research laboratory.

Test Principle

Spinxt FFPE DNA Extraction Kit is designed to extract DNA/RNA from formalin fixed paraffin-embedded tissue samples. FFPE samples are incubated in an optimized lysis buffer, which results in the release of RNA and precipitation of DNA. After centrifugation, the RNA-containing supernatant and DNA-containing pellet are then processed separately to purify the RNA and DNA. For RNA Purification, transfer RNA Lysate into an adsorption column and RNA is adsorbed on the membrane, while protein is not adsorbed and is removed with filtration. After washing-off the proteins and other impurities, RNA is finally eluted with low-salt buffer. For DNA Purification, transfer DNA Lysate to an adsorption column and DNA is adsorbed on the membrane, while protein is not adsorbed and is removed with filtration. After washing proteins and other impurities, DNA was finally eluted with low-salt buffer.

Summary

SpiNXT FFPE DNA Extraction Kit is solely designed for the purification of genomic DNA/RNA from formalin-fixed paraffin-embedded (FFPE) tissue sections. Fixing of tissues using formalin generates the cross-linking of the nucleic acids, proteins and also the process of embedding the tissue samples might lead to fragmentation of the nucleic acids over a period of time effecting the yield. This kit enables the partial reversing of the modification caused by formalin which results in high yield with good quality. This product is based on silica column purification without the use of phenol-chloroform extraction or alcohol precipitation and the system utilizes advantages of silica binding with a micro-spin format with minimal contamination from proteins and cell debris. The purified DNA/RNA can be used for various downstream applications including qPCR, mutation screening, microarray analysis, sequencing, southern blotting and SNP analysis.

Storage, Operating Conditions and Stability

- The kit has a shelf life of 18 months from the date of manufac turing.
- The test kit and its component are stable until the expiration date mentioned on the kit box.
- All the kit Components is shipped and stored at 15 °C to 25 °C.

Important Notes

- Yield of the DNA/RNA may vary depending upon the tissue type, quality of the FFPE tissue sections, source, the width of the slice, the age of the sample, fixation time, post-sampling delay before fixation, etc.
- Tissue sections can be stored at 4 °C or below for one year without any recognizable effects on the DNA/RNA yield and integrity.
- Avoid repeated freeze/thaw cycles of the samples.

Reagents Provided
Table 1a. (For 50 Tests)

Kit Contents	Kit Content Code	Kit Content Quantity G2MBR4-0652
Deparaffinization solution	G2MBR3-1907-1	1 X 60 ml
Buffer LP	G2MBR3-1908-1	1 X 15 ml
Buffer ATL	G2MBR3-1909-1	1 X 15 ml
Buffer RLC	G2MBR3-1910-1	1 X 15 ml
Buffer TLB	G2MBR3-1911-1	1 X 15 ml
Buffer W1	G2MBR3-1912-1	1 X 44 ml
Buffer W2	G2MBR3-1913-1	1 X 25 ml
Proteinase K	G2MBR3-1914-1	1 X 50 mg
Protease Dissolve Buffer	G2MBR3-1915-1	1 X 5 ml
Buffer AE	G2MBR3-1916-1	1 X 10 ml
RNase/DNase Water	G2MBR3-1917-1	1 X 10 ml

Consumables Provided
Table 1b. (For 50 Tests)

Kit Contents	Kit Content Quantity G2MBR4-0652
DNA Mini Column	1 X 50 Nos.
RNA Mini Column	1 X 50 Nos.
Collection Tubes	3 X 50 Nos.

Reagents Provided
Table 2a. (For 250 Tests)

Kit Contents	Kit Content Code	Kit Content Quantity G2MBR4-0653
Deparaffinization solution	G2MBR3-1907-2	1 X 300 ml
Buffer LP	G2MBR3-1908-2	1 X 75 ml
Buffer ATL	G2MBR3-1909-2	1 X 75 ml
Buffer RLC	G2MBR3-1910-2	1 X 75 ml
Buffer TLB	G2MBR3-1911-2	1 X 75 ml
Buffer W1	G2MBR3-1912-2	1 X 220 ml
Buffer W2	G2MBR3-1913-2	2 X 63 ml
Proteinase K	G2MBR3-1914-2	1 X 250 mg
Protease Dissolve Buffer	G2MBR3-1915-2	1 X 25 ml
Buffer AE	G2MBR3-1916-2	1 X 50 ml
RNase/DNase Water	G2MBR3-1917-2	1 X 50 ml

Consumables Provided
Table 2b. (For 250 Tests)

Kit Contents	Kit Content Quantity G2MBR4-0653
DNA Mini Column	2 X 125 Nos.
RNA Mini Column	2 X 125 Nos.
Collection Tubes	6 X 125 Nos.

Materials Required But Not Provided

- Water bath or Heating block
- Micropipettes (Adjustable)
- Disposable Barrier (Filter) tips
- 1.5/2 ml microcentrifuge tubes (DNase/RNase Free)
- Table top microcentrifuge
- Molecular biology grade ethanol (96-100 %)
- Personal protective equipment (aprons, goggles etc).
- Disposable Powder-free Gloves
- Vortexer

⚠ Instructions Before Use

- Preheat a water bath or heating block to 56 °C.
- Buffer W1 and Buffer W2 are supplied as concentrates. Before using for the first time, add the appropriate volume of molecular biology grade ethanol (96-100 %) as indicated on the bottle and shake thoroughly. Buffer W1 and Buffer W2 are stable for at least 18 months after the reconstitution with ethanol when stored closed at room temperature (15 °C to 25 °C).
- Add Protease Dissolve Buffer into absolute amount of Proteinase K as mentioned on the bottle label and store it at -20 °C.

Protocol

1) Using a scalpel, trim the excess paraffin from the sample block. Cut sections 10-20 µm thick.

2) Transfer 1-6 sections to 1.5/2 ml microcentrifuge tube. Add 700 µl Deparaffinization Solution to the sample. Vortex for 5 sec and centrifuge briefly to bring the sample to the bottom of the tube. Do not use more than six 10 µm sections of ~150 mm^2 surface area or three 20 µm sections of ~150 mm^2 surface area. If the sample surface has been exposed to air, discard the first 2-3 sections.

3) Incubate at 56 °C for 20 min and vortex vigorously for 15 sec to dissolve the paraffin completely. Centrifuge at full speed for 2 min. Remove the supernatant by pipetting without disturbing the pellet.

4) Centrifuge at 14,000 x*g* for 2 min. Aspirate and discard the supernatant carefully, do not disturb the pellet.

5) Resuspend the pellet by adding 180 µl Buffer LP and flicking the tube to loosen the pellet.

6) Add 20 µl Proteinase K and mix by vortexing. Incubate at 56 °C for 15 min. Depending on the sample material, the sample may not be completely lysed. This does not affect the procedure.

7) Incubate on ice for 3 min and centrifuge for 15 min at 20,000 x*g*.

8) Carefully transfer the supernatant, without disturbing the pellet, to a new 1.5/2 ml microcentrifuge tube for RNA purification as give in steps 9-18. Keep the pellet for DNA purification as given in steps 19-28. Depending on the amount and nature of the FFPE sample, the pellet may be very small or difficult to see. If the pellet is aspirated with the supernatant, allow the pellet to drop slowly to the bottom of the tube. The DNA-containing pellet can be stored for two hours at room temperature, for up to one day at 2 °C to 8 °C or for longer periods at -30 °C to -10 °C.

RNA Purification

9) Incubate the supernatant from step 7 at 80 °C for 15 min. This incubation step partially reverses formaldehyde modification of nucleic acids. Longer incubation times or higher incubation temperatures may result in more fragmented RNA.

10) Add 200 µl Buffer RLC, and mix thoroughly by vortexing.

11) Add 600 µl ethanol (96-100 %), and mix thoroughly again by vortexing.

12) Insert a RNA Mini Column I in a 2 ml Collection Tube.

13) Add up to 600 µl of the sample from step 10 to the Column. Centrifuge at 12,000 x*g* for 1 min at room temperature. Discard the filtrate and reuse the collection tube.

14) Repeat step 12 until all of the sample has been transferred to the column.

15) Add 500 µl Buffer W1 to the column, centrifuge at 12,000 x*g* for 1 min at room temperature. Discard the filtrate and reuse the collection tube.

16) Add 650 µl Buffer W2 to the column, Centrifuge at 12,000 x*g* for 1 min at room temperature. Discard the filtrate and reuse the collection tube.

17) Centrifuge the empty Column at 12,000 x*g* for 2 min at room temperature to dry the column matrix.

18) Transfer the Column to a clean 1.5/2 ml microcentrifuge tube. Add 30-50 µl RNase Free Water directly to the center of the column membrane. Leave the column undisturbed at room temperature for 2 min. Centrifuge at 12,000 x*g* for 1 min at room temperature. Store the RNA at -20 °C/-80 °C.

DNA Purification

19) Resuspend the pellet from step 7 in 180 µl Buffer ATL and add 20 µl Proteinase K. Mix by vortexing.

20) Incubate at 56 °C for 1 h. Incubate at 90 °C for 2 h. This incubation step partially reverses the formaldehyde modification of nucleic acids. Longer incubation time or higher incubation temperature may result in more fragmented DNA.

21) Add 200 µl Buffer TLB, and mix thoroughly by vortexing.

22) Add 200 µl molecular biology grade ethanol (96-100 %), and mix thoroughly again by vortexing.

23) Insert a DNA Mini Column in a 2 ml Collection Tube.

24) Add the sample from Step 21 to the Column. Centrifuge at 12,000 x*g* for 1 min at room temperature. Discard the filtrate and reuse collection tube.

25) Add 500 µl Buffer W1 to the column, centrifuge at 12,000 x*g* for 1 min at room temperature. Discard the filtrate and reuse the collection tube.

26) Add 650 µl Buffer W2 to the column, Centrifuge at 12,000 x*g* for 1 min at room temperature. Discard the filtrate and reuse the collection tube.

27) Centrifuge the empty Column at 12,000 *xg* for 2 min at room temperature to dry the column matrix.

28) Transfer the Column into a clean 1.5/2 ml microcentrifuge tube. Add 20-50 µl Buffer AE directly to the center of the column membrane. Leave the column undisturbed at room temperature for 3 min. Centrifuge at 12,000 x*g* for 1 min at room temperature. Store the DNA at -20 °C/-80 °C.

Symbols for Use in the Labeling	
Symbols	**Definition**
	KEEP AWAY FROM SUNLIGHT
	TEMPERATURE LIMIT
	RESEARCH USE ONLY
	UPWARD
	CONSULT INSTRUCTIONS FOR USE
	BATCH CODE
	CATALOGUE NUMBER
	USE BY DATE
	DATE OF MANUFACTURE
	MANUFACTURER
	CONTAINS SUFFICIENT FOR <n> TESTS
	CAUTION
	DO NOT USE IF PACKAGE IS DAMAGED

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