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FFPE DNA Purification Kit

Product Insert

Product # 47400

Norgen's FFPE DNA Purification Kit provides a rapid method for the isolation and purification of genomic DNA from formalin-fixed paraffin-embedded (FFPE) tissue samples. Using formalin to fix tissues leads to crosslinking of the nucleic acids and proteins, and the process of embedding the tissue samples can also lead to fragmentation of the nucleic acids over time. Norgen's FFPE DNA Purification Kit provides conditions that allow for the partial reversing of the formalin modifications, resulting in a high quality and yield of nucleic acids. The DNA is purified from other cellular components without the use of phenol or chloroform. The purified genomic DNA is of the highest integrity, and can be used in a number of downstream applications including qPCR, mutation screening, microarray analyses, sequencing, Southern blotting and SNP analysis.

Norgen's Purification Technology

Purification is based on spin column chromatography as the separation matrix. The DNA is preferentially purified from other cellular components without the use of phenol or chloroform. The process first involves deparaffinization of the FFPE samples through a series of xylene and ethanol washes. Next, the FFPE samples are digested with the provided Proteinase K, RNase and Digestion Buffer A using an incubation time which is specific for the recovery of DNA (please see the flow chart on page 3). Buffer RL and ethanol are then added to the lysate, and the solution is loaded onto a spin-column. Norgen's column binds nucleic acids in a manner that depends on ionic concentrations, thus only the DNA will bind to the column while the contaminants will be removed in the flowthrough or retained on the top of the resin. The bound DNA is then washed with the provided Wash Solution A in order to remove any impurities, and the purified DNA is eluted with the Elution Buffer B.

Specifications

Kit Specifications	
Maximum Column Binding Capacity (gDNA)	*10 μg
Maximum Column Loading Volume	650 μL
Size of DNA Purified	All sizes > 80 bps
Maximum Amount of Starting Material	5 sections ≤20 μm thick 25 mg of unsectioned block

***** The column capacity is 15 μg for intact, large molecular weight gDNA. More than of 10 μg DNA may be recovered for DNA that is more fragmented which is common for FFPE samples

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. The Proteinase K should be stored at -20°C upon arrival and after reconstitution. This kit is stable for 1 year after the date of shipment.

Advantages

- Fast and easy processing using rapid spin-column format
- High yields of gDNA
- No phenol or chloroform extractions

Kit Components

Component	Product # 47400 (50 preps)
Digestion Buffer A	25 mL
Buffer RL	30 mL
Wash Solution A	38 mL
Elution Buffer B	15 mL
Proteinase K	12 mg
RNase A	1 tube
gDNA Purification Micro Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
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Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

The **Buffer RL** contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

Customer-Supplied Reagents and Equipment

You must have the following in order to use the FFPE DNA Purification Kit:

- Benchtop microcentrifuge
- 96 - 100% ethanol
- Xylene, histological grade
- 55°C Incubator
- 90°C Incubator

Procedures

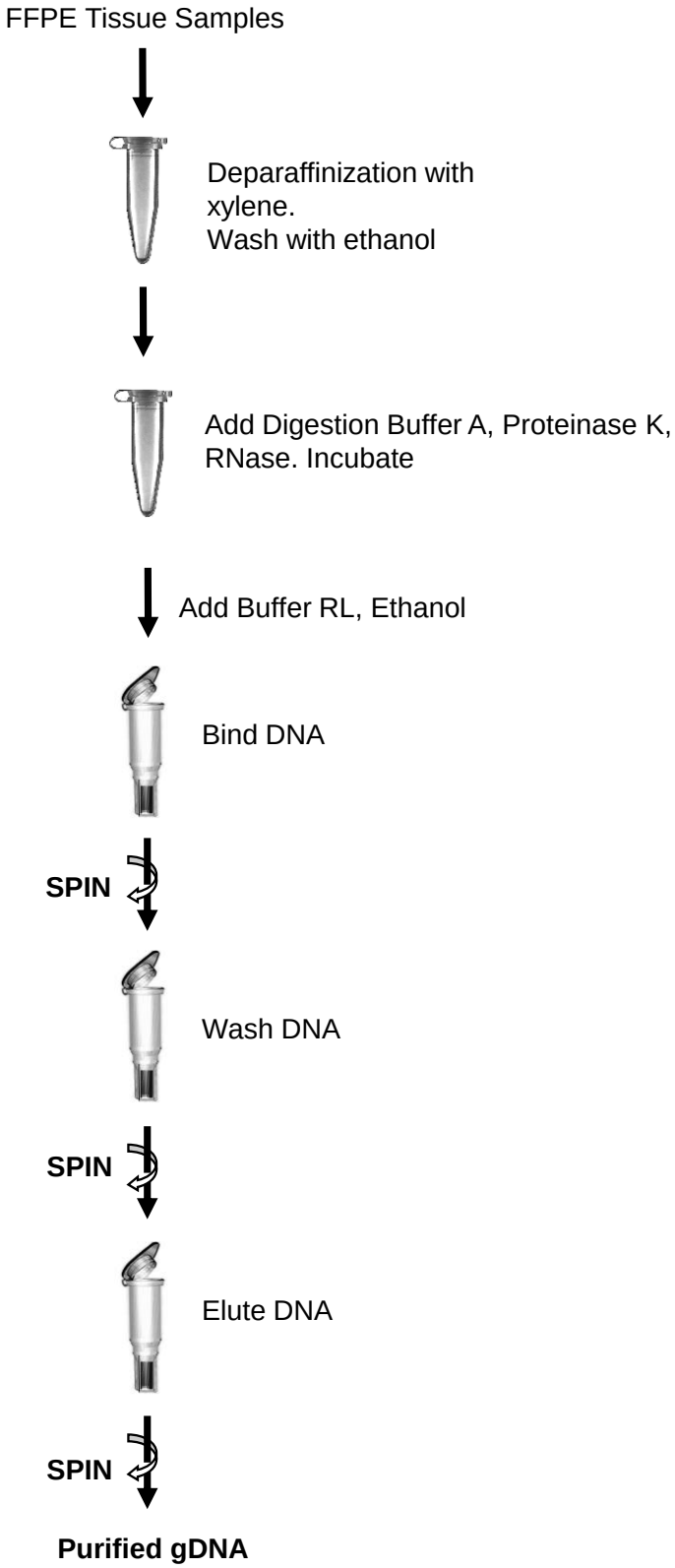
All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

Flowchart

Procedure for Purifying DNA using Norgen's FFPE DNA Purification Kit



Notes Prior to Use

- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~ 14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use.
- Reconstitute the **Proteinase K** in 600 µL of molecular biology grade water, aliquot into small fractions and store the unused portions at -20°C until needed.
- Prepare a working concentration of the **Wash Solution A** by adding 90 mL of 96 – 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution A**. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- The maximum recommended input is five sections of ≤ 20 µm thick. Alternatively, an unsectioned block of up to 25 mg may be used.
- It is important to obtain sections from the interior of an FFPE block in order to minimize DNA damage by oxidation.

1. Deparaffinization

a. Cut sections up to 20 µm thick from the interior of an FFPE tissue block using a microtome. Trim off any excess paraffin.

   Note: Alternatively, from an FFPE block, cut out up to 25 mg of unsectioned core. Trim off any excess paraffin. Grind the sample into fine powder using liquid nitrogen.

b. Transfer the sections or ground block into a Nuclease-free microcentrifuge tube.
c. Add 1 mL of xylene to the sample. Mix by vortexing.
d. Incubate at 50°C for 5 minutes.
e. Centrifuge the sample at 14,000 x g (~ 14,000 RPM) for 2 minutes.
f. Carefully remove the xylene without dislodging the pellet.
g. Add 1 mL of 96 - 100 % ethanol. Mix by vortexing.
h. Centrifuge the sample at 14,000 x g (~ 14,000 RPM) for 2 minutes.
i. Carefully remove the ethanol without dislodging the pellet.
j. Repeat Step **1g** to Step **1i** for a second time.
k. Air dry the pellet for about 10 minutes at room temperature.

   Note: It is important to remove the ethanol completely.

l. Proceed to Step 2. **Lysate Preparation**

2. Lysate Preparation

a. Add 300 µL of **Digestion Buffer A** and 10 µL of the reconstituted Proteinase K and 1 µL RNase to the sample. Mix by vortexing

b. Incubate at 55°C for 1 hour, followed by 90°C for 1 hour. Vortex to mix occasionally.

   Note: Most tissue samples will be digested or clarified within the time indicated. If significant amount of visible debris remains, centrifuge the samples at 14,000 x g (~ 14,000 RPM) for 2 minutes and transfer the supernatant to a new microcentrifuge tube.

c. Add 300 µL of **Buffer RL**. Vortex to mix.
d. Add 250 µL of 96 – 100 % ethanol. Vortex to mix.

3. Binding DNA to Column

a. Assemble a gDNA Purification Micro Column with one of the provided collection tubes.
b. Apply up to 600 µL of the clarified lysate with the ethanol (from **Step 2**) onto the column and centrifuge for 1 minute.
c. Discard the flowthrough. Reassemble the spin column with its collection tube.
d. Repeat Step 3b and 3c until all lysate has passed through the column.

4. Column Wash

a. Apply 400 µL of **Wash Solution A** to the column and centrifuge for 1 minute.

Note: Ensure the entire Wash Solution A has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Apply 400 µL of **Wash Solution A** to the column and centrifuge for 1 minute.
d. Discard the flowthrough and reassemble the spin column with its collection tube
e. Wash column a third time by adding another 400 µL of **Wash Solution A** and centrifuging for 1 minute.
f. Discard the flowthrough and reassemble the spin column with its collection tube.
g. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

5. DNA Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 20 - 50 µL of **Elution Buffer B** to the column. Incubate the assembly at room temperature for 1 minute.
c. Centrifuge for 1 minute **14,000 x *g* (~14,000 RPM).** Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.

Note: For maximum DNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 5b** and **5c**).

6. Storage of DNA

The purified DNA may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

Related Products	**Product #**
FFPE RNA Purification Kit	25300
Cytoplasmic & Nuclear RNA Purification Kit	21000
microRNA Purification Kit	21300
100b RNA Ladder	15002
1kb RNA Ladder	15003
FFPE RNA/DNA Purification Plus Kit	54300

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor DNA Recovery	Incomplete lysis of cells or tissue	Ensure that the appropriate amount of Digestion Buffer A with Proteinase K added was used. Increase the incubation time.
	Column has become clogged	Do not exceed the recommended amounts of starting materials. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. See also “Clogged Column” below.
	An alternative elution solution was used	It is recommended that the Elution Buffer B supplied with this kit be used for maximum DNA recovery.
	Ethanol or Buffer RL was not added to the lysate	Ensure that the appropriate amount of ethanol and Buffer RL is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution A	Ensure that 90 mL of 96 – 100 % ethanol is added to the supplied Wash Solution A prior to use.
	Low DNA content in cells or tissues used	Different tissues and cells have different DNA contents, and thus the expected yield of DNA will vary greatly from these different sources. Please check literature to determine the expected DNA content of your starting material.
Clogged Column	Insufficient solubilization of cells or tissues	Ensure that the appropriate amount of lysis buffer was used for the amount of cells or tissue.
	Maximum number of cells or amount of tissue exceeds kit specifications	Refer to specifications to determine if amount of starting material falls within kit specifications
	Clarified lysate was not used for the binding step	Ensure that after the lysis step the sample is centrifuged if a significant amount of debris is present, and that only the clarified lysate is used in subsequent steps.
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°C may cause precipitates to form that can cause the columns to clog.

Problem	Possible Cause	Solution and Explanation
DNA is Degraded	FFPE sample is old	The quality of DNA purified may drastically decrease in old samples. For best performance, freshly prepared samples are highly recommended.
DNA does not perform well in downstream applications	DNA was not washed 3 times with the provided Wash Solution A	Traces of salt from the binding step may remain in the sample if the column is not washed 3 times with Wash Solution A. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
	Formalin crosslink was not completely reversed	Ensure the sufficient incubation at 50°C and 90°C is performed in Step **2b**.

Technical Support
Contact our Technical Support Team between the hours of 9:00 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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