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FFPE RNA/DNA Purification Kit — Product Insert

Product # 25000

Norgen's FFPE RNA/DNA Purification Kit provides a rapid method for the isolation and purification of total RNA (including microRNA) and genomic DNA from formalin-fixed paraffin-embedded (FFPE) tissue samples. Alternatively, the kit can be used to isolate total RNA alone, or genomic DNA alone from 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 RNA/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 kit is able to purify all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA), depending on the age of the FFPE tissue as the degree of fragmentation of the RNA will increase over time. The RNA is purified from other cellular components without the use of phenol or chloroform. The purified RNA and genomic DNA are of the highest integrity, and can be used in a number of downstream applications including qPCR, qRT-PCR, reverse transcription PCR, primer extension, mutation screening, expression array assays, microarray analyses, sequencing, Southern blotting and SNP analysis.

Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The nucleic acids are 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 and Digestion Buffer using an incubation time which is specific for the recovery of either RNA/DNA or DNA (please see the flow chart on page 4). Binding Solution and ethanol are then added to the lysate, and the solution is loaded onto a spin-column. Norgen's resin binds nucleic acids in a manner that depends on ionic concentrations, thus only the RNA and/or DNA will bind to the column while the contaminants will be removed in the flowthrough or retained on the top of the resin. At this point, any traces of genomic DNA can be digested allowing for pure RNA samples to be isolated. Alternatively, traces of the RNA may be digested at this point if a pure sample of genomic DNA is required instead. The bound nucleic acid is then washed with the provided Wash Solution in order to remove any impurities, and the purified nucleic acid is eluted with the Elution Buffer.

Specifications

Kit Specifications	
Column Binding Capacity (RNA)	50 μg
Column Binding Capacity (gDNA)	15 μg
Maximum Column Loading Volume	600 μL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Maximum Amount of Starting Material	5 sections ≤20 μM thick 25 mg of unsectioned block

Advantages

- Fast and easy processing using rapid spin-column format
- High yields of RNA and/or gDNA
- Isolate total RNA, from large rRNA down to microRNA (miRNA)
- No phenol or chloroform extractions
- Versatile procedure to isolate either high quality total RNA and gDNA, or total RNA or genomic DNA

Kit Components

Component	Product # 25000 (50 preps)
Digestion Buffer	20 mL
Binding Solution	20 mL
Enzyme Incubation Buffer	6 mL
Wash Solution	22 mL
Elution Buffer	20 mL
Proteinase K	12 mg
Mini Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. The Proteinase K should be stored in aliquots at -20°C upon reconstitution. These reagents should remain stable for at least 2 years in their unopened containers.

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*.

Blood of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with whole blood.

Customer-Supplied Reagents and Equipment

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

For All Protocols

- Benchtop microcentrifuge
- 95 - 100% ethanol
- Xylene, histological grade
- β-mercaptoethanol (optional)
- DNase I (optional)
- RNase (optional)

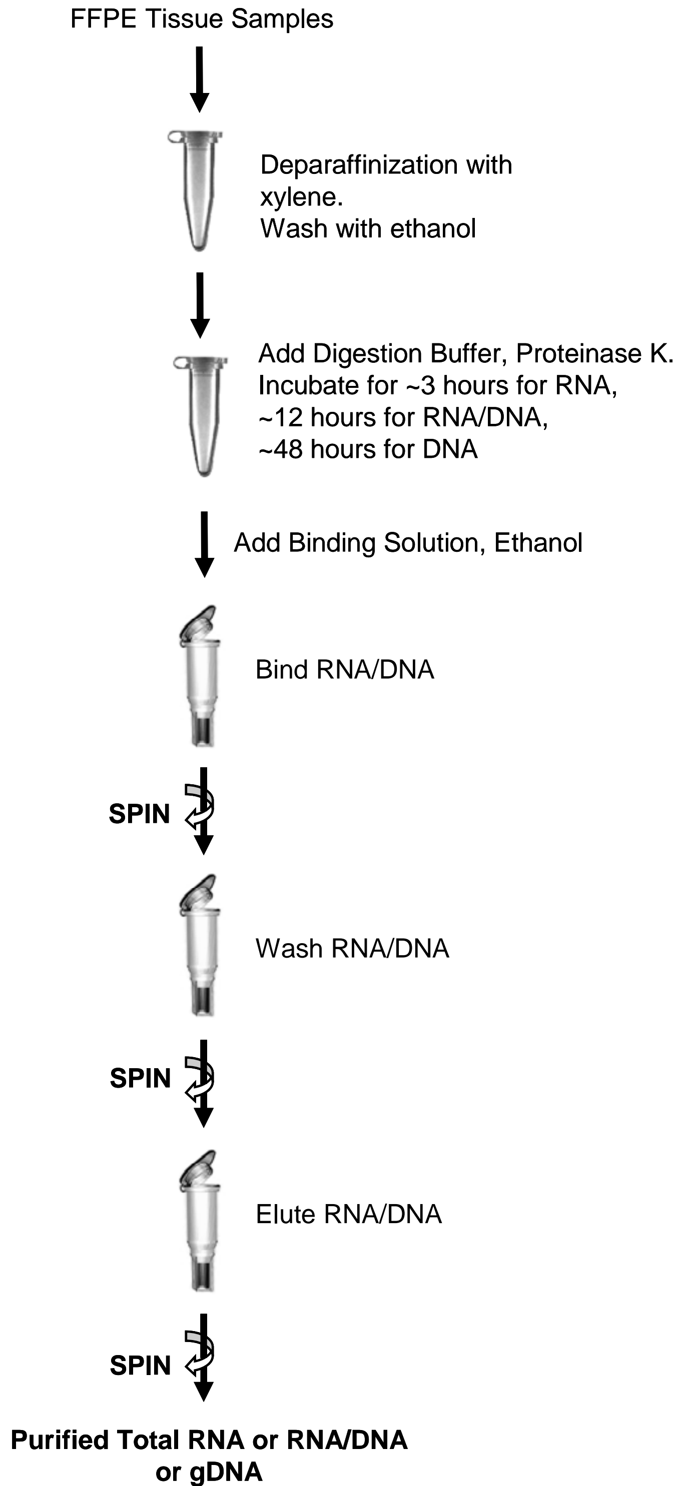
Working with RNA

RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these enzymes.

- The RNA area should be located away from microbiological work stations
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination
- There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only
- All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water
- Clean all surfaces with commercially available RNase decontamination solutions
- When working with purified RNA samples, ensure that they remain on ice during downstream applications

Flowchart

Procedure for Purifying Total RNA using Norgen's FFPE RNA/DNA Purification Kit



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.

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** by adding 50 mL of 95% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution**. This will give a final volume of 72 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- **Optional:** For sensitive downstream applications or target transcripts of low quantity, add 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of **Binding Solution** required. β-mercaptoethanol is toxic and should be dispensed in a fume hood. Alternatively, the **Binding Solution** can be used as provided.
- The maximum recommended input is five sections of $\leq$ 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 RNA damage by oxidation.
- It is important to work quickly during this procedure.

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 an RNase-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 95 - 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 and 10 μL of the reconstituted Proteinase K to the sample. Mix by vortexing
b. Incubate at 50°C for 1.5 to 48 hours. Vortex to mix occasionally.

   Note: Most tissue samples will be digested or clarified within 1 to 3 hours. Additional incubation is required to reverse the effect of formalin fixation. 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. For RNA, the optimal incubation time is 3 hours. As little as 1 hour incubation may be used although the isolated RNA may not perform as well in downstream applications (for example, a loss of 1-2 C_T units may occur for qRT-PCR). For DNA, the optimal incubation time is 24 - 48 hours, and a minimum incubation of 12 hours is required. For the isolation of both DNA and RNA, incubate the samples for 12 – 24 hours. Note that prolonged incubation beyond 12 hours may result in some degradation of RNA.

c. Add 300 μL of **Binding Solution**. Vortex to mix.
d. Add 600 μL of 95 – 100 % ethanol. Vortex to mix.

3. Binding RNA to Column

a. Assemble a 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.

Optional Step:

Norgen's FFPE RNA/DNA Purification Kit isolates total RNA with minimal amounts of genomic DNA contamination when a 3 hour incubation time is used during the lysate preparation. However, an optional **On-Column DNA Removal Protocol** is provided in Appendix A for maximum removal of residual DNA that may affect sensitive downstream applications. This step should be performed at this point in the protocol. Alternatively if RNA-free genomic DNA is to be isolated, the optional **On-Column RNA Removal Protocol** can be performed at this point (Appendix B).

4. Column Wash

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

Note: Ensure the entire wash solution 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** 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** 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. Nucleic Acid Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 150 μL of **Nucleic Acid Elution Buffer** to the column.

Note: If only RNA is being isolated, reduce the volume of **Nucleic Acid Elution Buffer** to 50 μL.

c. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by a 1 minute spin at **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 nucleic acid recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 5b** and **5c**).

6. Storage of DNA and RNA

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

Appendix A

Protocol for Optional On-Column DNA Removal

Notes Prior to Use

- This optional step is carried out if genomic DNA-free RNA is required.
- Prepare a DNase I mixture by adding 10 units of RNase-free DNase I to 100 µL of **Enzyme Incubation Buffer** for each isolation.

a. Apply 400 µL of **Wash Solution** to the column and centrifuge for 2 minutes. Discard the flowthrough.

Note: Ensure the entire wash solution 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. Apply 100 µL of **Enzyme Incubation Buffer** mix containing the RNase-free DNase I to the column.

c. Centrifuge for 30 seconds at **200 x *g* (~2,000 RPM)** to allow half of the Enzyme Reaction Buffer mix to pass through the column. Alternatively, if the centrifuge used has no speed adjustment, centrifuge for 5 seconds at 14000 x g (~14000 RPM).
d. Incubate the whole unit at room temperature for 15 minutes.
e. Proceed to Step **4c** without further centrifugation.

Appendix B

Protocol for Optional On-Column RNA Removal

Notes Prior to Use

- This optional step is carried out if genomic DNA-free RNA is required.
- Prepare an RNase mixture by adding 10 units of RNase to 100 µL of **Enzyme Incubation Buffer** for each isolation.

a. Apply 400 µL of **Wash Solution** to the column and centrifuge for 2 minutes. Discard the flowthrough.

Note: Ensure the entire wash solution 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. Apply 100 µL of **Enzyme Incubation Buffer** containing the RNase to the column.

c. Centrifuge for 30 seconds at **200 x *g* (~2,000 RPM)** to allow half of the Enzyme Reaction Buffer mix to pass through the column. Alternatively, if the centrifuge used has no speed adjustment, centrifuge for 5 seconds at 14000 x g (~14000 RPM).
d. Incubate the whole unit at room temperature for 15 minutes.
e. Proceed to Step **4c** without further centrifugation.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Incomplete lysis of cells or tissue	Ensure that the appropriate amount of Digestion Buffer 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 supplied with this kit be used for maximum RNA recovery.
	Ethanol or Binding Solution was not added to the lysate	Ensure that the appropriate amount of ethanol and Binding Solution is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution	Ensure that 50 mL of 95% ethanol is added to the supplied Wash Solution prior to use.
	Low RNA content in cells or tissues used	Different tissues and cells have different RNA contents, and thus the expected yield of RNA will vary greatly from these different sources. Please check literature to determine the expected RNA 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
RNA is Degraded	FFPE sample is old	The quality of RNA purified may drastically decrease in old samples. For best performance, freshly prepared samples are highly recommended.
	RNase contamination	RNases may be introduced during the use of the kit. Ensure proper procedures are followed when working with RNA. Please refer to "*Working with RNA"* at the beginning of this user guide.
	Procedure not performed quickly enough	In order to maintain the integrity of the RNA, it is important that the procedure be performed quickly. This is especially important for the Cell Lysate Preparation Step in the Animal Tissue protocol, since the RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized.
	Improper storage of the purified RNA	For short term storage RNA samples may be stored at –20°C for a few days. It is recommended that samples be stored at –70°C for longer term storage.
	Prolonged incubation at 50°C	In order to reverse formalin crosslinks, an incubation at 50°C is required which may lead to degraded RNA.
	Starting material may have a high RNase content	For starting materials with high RNAase content, it is recommended that β-mercaptoethanol be added to the Lysis Solution.
Nucleic acids does not perform well in downstream applications	Nucleic acids were not washed 3 times with the provided Wash Solution	Traces of salt from the binding step may remain in the sample if the column is not washed 3 times with Wash Solution. 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 is performed in Step **2b**. The optimal time for RNA is 3 hours and for DNA is 12 - 24 hours.
Genomic DNA contamination	Large amounts of starting material used	Perform RNAse-free DNaseI digestion on the RNA sample after elution to remove genomic DNA contamination.

Related Products	Product #
RNA/Protein Purification Kit	23000
RNA/DNA/Protein Purification Kit	23500
Cytoplasmic & Nuclear RNA Purification Kit	21000
Leukocyte RNA Purification Kit	21200
microRNA Purification Kit	21300
100b RNA Ladder	15002
1kb RNA Ladder	15003

Technical Support
Contact our Technical Support Team between the hours of 8:30 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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