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

<table>
<thead>
<tr>
<th>Kit Specifications</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum Column Binding Capacity (RNA)</td>
<td>50 µg</td>
</tr>
<tr>
<td>Maximum Column Binding Capacity (gDNA)</td>
<td>15 µg</td>
</tr>
<tr>
<td>Maximum Column Loading Volume</td>
<td>650 µL</td>
</tr>
<tr>
<td>Size of RNA Purified</td>
<td>All sizes, including small RNA (&lt;200 nt)</td>
</tr>
<tr>
<td>Maximum Amount of Starting Material</td>
<td>5 sections &lt;20 µM thick 25 mg of unsectioned block</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Component</th>
<th>Product # 25000 (50 preps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestion Buffer</td>
<td>20 mL</td>
</tr>
<tr>
<td>Binding Solution</td>
<td>20 mL</td>
</tr>
<tr>
<td>Enzyme Incubation Buffer</td>
<td>6 mL</td>
</tr>
<tr>
<td>Wash Solution</td>
<td>22 mL</td>
</tr>
<tr>
<td>Elution Buffer</td>
<td>20 mL</td>
</tr>
<tr>
<td>Proteinase K</td>
<td>12 mg</td>
</tr>
<tr>
<td>Mini Spin Columns</td>
<td>50</td>
</tr>
<tr>
<td>Collection Tubes</td>
<td>50</td>
</tr>
<tr>
<td>Elution tubes (1.7 mL)</td>
<td>50</td>
</tr>
<tr>
<td>Product Insert</td>
<td>1</td>
</tr>
</tbody>
</table>

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. These reagents should remain stable for at least 1 year 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:

- Benchtop microcentrifuge
- 95 - 100% ethanol
- Xylene, histological grade
- β-mercaptoethanol (optional)
- DNase I (optional)
- RNase (optional)
- 55 °C Incubator
- 80 °C Incubator (for RNA)
- 90 °C Incubator (for DNA)
Flowchart
Procedure for Purifying Total RNA using Norgen’s FFPE RNA/DNA Purification Kit

FFPE Tissue Samples

- Deparaffinization with xylene.
  Wash with ethanol

- Add Digestion Buffer, Proteinase K.
  - RNA incubate at 55°C for 15 minutes followed by 80°C for 15 minutes.
  - DNA incubate at 55°C for 1 hour followed by 90°C for 1 hour.

- Add Binding Solution, Ethanol

- Bind RNA/DNA

- SPIN

- Wash RNA/DNA

- SPIN

- Elute RNA/DNA

- SPIN

- Purified Total RNA or RNA/DNA or gDNA
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

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:

\[
RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5}) \cdot r}}
\]

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 ≤ 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 1f 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. **For RNA:** Incubate at 55°C for 15 minutes, followed by 80°C for 15 minutes. Vortex to 
      mix occasionally. Proceed to Step 2d.  
      **Note:** Do not exceed 15 minutes of incubation at 80°C as this will increase RNA 
      fragmentation.  
   c. **For DNA:** Incubate at 55°C for 1 hour, followed by 90°C for 1 hour. Vortex to mix 
      occasionally. Proceed to Step 2d.  
   d. Add 300 µL of Binding Solution. Vortex to mix.  
   e. **For RNA:** Add 600 µL of 95 – 100 % ethanol. **For DNA:** Add 250 µL of 95 – 100 % 
      ethanol. Vortex to mix. 

3. Binding RNA or DNA 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. It is recommended that Norgen’s RNase-Free 
DNase I Kit (Product # 25710) be used for this step. 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 15 μL of Norgen’s RNase-Free DNase I (Product #25710) to 100 μL of Enzyme Incubation Buffer for each isolation.
- If an alternate DNase is to be used, add 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 and centrifuge at 14,000 x g (~14,000 RPM) for 1 minute.
Note: Ensure that the entire volume of DNase I mix passes through the column. If needed, spin at 14,000 x g for an additional minute.

c. After the centrifugation in Step b, pipette the flowthrough that is present in the collection tube back onto the top of the column.

Note: Ensure that Step c is performed in order to ensure maximum DNase activity and to obtain maximum yields of RNA, in particular for small RNA species.

d. Incubate 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 RNA-free genomic DNA 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 mix containing RNase to the column and centrifuge at 14,000 x g (~14,000 RPM) for 1 minute.

Note: Ensure that the entire volume of RNase mix passes through the column. If needed, spin at 14,000 x g for an additional minute.

c. After the centrifugation in Step b, pipette the flowthrough that is present in the collection tube back onto the top of the column.

d. Incubate at room temperature for 15 minutes.
e. Proceed to Step 4c without further centrifugation.

Related Products

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Product #</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNase-Free DNase I Kit</td>
<td>25710</td>
</tr>
<tr>
<td>FFPE RNA Purification Kit</td>
<td>25300</td>
</tr>
<tr>
<td>RNA/Protein Purification Kit</td>
<td>23000</td>
</tr>
<tr>
<td>RNA/DNA/Protein Purification Kit</td>
<td>23500</td>
</tr>
<tr>
<td>Cytoplasmic &amp; Nuclear RNA Purification Kit</td>
<td>21000</td>
</tr>
<tr>
<td>microRNA Purification Kit</td>
<td>21300</td>
</tr>
<tr>
<td>100b RNA Ladder</td>
<td>15002</td>
</tr>
<tr>
<td>1kb RNA Ladder</td>
<td>15003</td>
</tr>
</tbody>
</table>

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.
## Troubleshooting Guide

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution and Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor RNA Recovery</td>
<td>Incomplete lysis of cells or tissue</td>
<td>Ensure that the appropriate amount of Digestion Buffer with Proteinase K added was used. Increase the incubation time.</td>
</tr>
<tr>
<td></td>
<td>Column has become clogged</td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>An alternative elution solution was used</td>
<td>It is recommended that the Elution Buffer supplied with this kit be used for maximum RNA recovery.</td>
</tr>
<tr>
<td></td>
<td>Ethanol or Binding Solution was not added to the lysate</td>
<td>Ensure that the appropriate amount of ethanol and Binding Solution is added to the lysate before binding to the column.</td>
</tr>
<tr>
<td></td>
<td>Ethanol was not added to the Wash Solution</td>
<td>Ensure that 50 mL of 95% ethanol is added to the supplied Wash Solution prior to use.</td>
</tr>
<tr>
<td></td>
<td>Low RNA content in cells or tissues used</td>
<td>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.</td>
</tr>
<tr>
<td>Clogged Column</td>
<td>Insufficient solubilization of cells or tissues</td>
<td>Ensure that the appropriate amount of lysis buffer was used for the amount of cells or tissue.</td>
</tr>
<tr>
<td></td>
<td>Maximum number of cells or amount of tissue exceeds kit specifications</td>
<td>Refer to specifications to determine if amount of starting material falls within kit specifications</td>
</tr>
<tr>
<td></td>
<td>Clarified lysate was not used for the binding step</td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>Centrifuge temperature too low</td>
<td>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.</td>
</tr>
<tr>
<td>Problem</td>
<td>Possible Cause</td>
<td>Solution and Explanation</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>----------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>RNA is Degraded</td>
<td>FFPE sample is old</td>
<td>The quality of RNA purified may drastically decrease in old samples. For best performance, freshly prepared samples are highly recommended.</td>
</tr>
<tr>
<td></td>
<td><strong>RNase contamination</strong></td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>Procedure not performed quickly enough</td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>Improper storage of the purified RNA</td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td><strong>Prolonged incubation at 50°C</strong></td>
<td>In order to reverse formalin crosslinks, an incubation at 50°C is required which may lead to degraded RNA.</td>
</tr>
<tr>
<td></td>
<td>Starting material may have a high RNase content</td>
<td>For starting materials with high RNAase content, it is recommended that β-mercaptoethanol be added to the Lysis Solution.</td>
</tr>
<tr>
<td>Nucleic acids does not perform well in downstream applications</td>
<td>Nucleic acids were not washed 3 times with the provided Wash Solution</td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td><strong>Ethanol carryover</strong></td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td><strong>Formalin crosslink was not completely reversed</strong></td>
<td>Ensure the samples are incubated at the proper temperature and for the proper amount of time as indicated in Step 2b.</td>
</tr>
<tr>
<td>Genomic DNA contamination</td>
<td>Large amounts of starting material used</td>
<td>Perform RNase-free DNasel digestion on the RNA sample after elution to remove genomic DNA contamination. It is recommended that Norgen’s RNase-Free DNase I be used for this step (Product # 25710).</td>
</tr>
</tbody>
</table>