CleanAll DNA/RNA Clean-up and Concentration Micro Kit

Product # 23800

Norgen’s CleanAll DNA/RNA Clean-up and Concentration Micro Kit provides a rapid method for the purification, cleanup and concentration of DNA or RNA from different isolation methods or upstream applications. This kit purifies all sizes of DNA, from small PCR products, native or linearized plasmids, to genomic DNA. The kit can be used as an alternative to organic extraction and ethanol precipitation to clean up various enzymatic reactions. It effectively removes PCR by-products including primers, dimers, enzymes, and unincorporated nucleotides. The purified DNA is fully compatible with restriction enzyme digestion, ligation, labeling, PCR and sequencing.

This kit also purifies RNA from phenol/guanidine-based protocols or from various upstream enzymatic reactions such as DNase treatment, labeling and in vitro transcription. The kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA). The RNA is preferentially purified from other reaction components such as proteins and nucleotides, without the use of phenol or chloroform. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including end-point or quantitative reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays. Norgen’s CleanAll DNA/RNA Clean-up and Concentration Micro Kit also provides a protocol for the rapid removal of endotoxins from previously purified DNA down to 0.1 EU/μg DNA or less. Endotoxins, also known as lipopolysaccharides, are cell-membrane components of Gram-negative bacteria such as E. coli. Endotoxins are released during the lysis step of plasmid purification and significantly reduce transfection efficiencies in endotoxin sensitive cell lines. Therefore, the removal of endotoxins from plasmid preparations is often necessary prior to the use of the DNA in downstream applications.

Norgen’s Purification Technology

Purification is based on spin column chromatography using Norgen’s proprietary resin as the separation matrix. The DNA or RNA is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. The process involves first mixing the nucleic acid samples or enzymatic reactions containing DNA or RNA with Binding Buffer H (please see the flow chart on page 5). For RNA purification, ethanol is then added and the mixture is loaded onto a spin-column. Norgen’s resin binds nucleic acids in a manner that depends on ionic concentrations. Thus only the nucleic acids will bind to the column, while the contaminating proteins or nucleotides will be removed in the flowthrough. The bound nucleic acid is then washed two times with the provided Wash Solution K in order to remove any remaining impurities. The purified DNA or RNA is eluted with Elution Buffer L. The purified DNA or RNA is of the highest integrity, and can be used in a number of downstream applications.
### Specifications

<table>
<thead>
<tr>
<th>Kit Specifications</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Column Binding Capacity</td>
<td>50 µg for RNA, 10 µg for DNA</td>
</tr>
<tr>
<td>Maximum Column Loading Volume</td>
<td>600 µL</td>
</tr>
<tr>
<td>Size of RNA Purified</td>
<td>All sizes, including small RNA (&lt;200 nt)</td>
</tr>
<tr>
<td>Size of DNA Purified</td>
<td>≥ 100 bp</td>
</tr>
<tr>
<td>Maximum Amount of Starting Material:</td>
<td>50 µg of RNA, 10 µg of DNA</td>
</tr>
<tr>
<td>Time to Complete 10 Purifications</td>
<td>20 minutes</td>
</tr>
<tr>
<td>Minimum Elution Volume</td>
<td>20 µL</td>
</tr>
<tr>
<td>Average Recovery</td>
<td>≥ 90% for RNA</td>
</tr>
<tr>
<td></td>
<td>≥ 90% for DNA from 100 bp to 10 kbp</td>
</tr>
<tr>
<td></td>
<td>≥ 75% for DNA ≥ 10 kbp</td>
</tr>
</tbody>
</table>

### Kit Applications:

#### Applications Requiring DNA Cleanup

<table>
<thead>
<tr>
<th>Applications Requiring DNA Cleanup</th>
<th>Contaminants Removed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Restriction Enzyme Digestion</td>
<td>Restriction enzymes, salts</td>
</tr>
<tr>
<td>DNA Modifications (Klenow, T4 Polynucleotide Kinase, Alkaline Phosphatase)</td>
<td>DNA modification enzymes, nucleotides, salts</td>
</tr>
<tr>
<td>PCR Reactions</td>
<td>DNA polymerases, nucleotides, salts, primers, mineral oils</td>
</tr>
<tr>
<td>DNA Probe Labeling (Radioactive, Fluorescent or Digoxigenin)</td>
<td>DNA modification enzymes or polymerase, nucleotides, label or coupling reagent, salts, primers</td>
</tr>
<tr>
<td>Ligation</td>
<td>Ligase, nucleotides, salts</td>
</tr>
<tr>
<td>Transfection, Microinjection</td>
<td>Endotoxin</td>
</tr>
<tr>
<td>DNA Staining (Including CsCl-gradients)</td>
<td>Intercalating dyes such as ethidium bromide and SYBR Green, salts</td>
</tr>
</tbody>
</table>

#### Applications Requiring RNA Cleanup

<table>
<thead>
<tr>
<th>Applications Requiring RNA Cleanup</th>
<th>Contaminants Removed</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNase treatment</td>
<td>DNase, salts</td>
</tr>
<tr>
<td><em>In vitro</em> transcription</td>
<td>DNA template, nucleotides, enzymes, salts</td>
</tr>
<tr>
<td>RNA Probe Labeling (Radioactive, Fluorescent or Digoxigenin)</td>
<td>RNA polymerase, nucleotides, salts, label or coupling reagent</td>
</tr>
<tr>
<td>Organic-based RNA Isolation (such as Trizol-based lysate)</td>
<td>Phenol, chloroform</td>
</tr>
</tbody>
</table>
Advantages

- Versatile performance for cleaning up all nucleic acids
- Fast and easy processing using rapid spin-column format
- Suitable for all sizes of RNA, from large rRNA down to microRNA (miRNA)
- Suitable for all sizes of DNA, from large gDNA down to small PCR fragments
- Optional endotoxin removal
- No phenol or chloroform extractions
- Cleanup nucleic acid from a variety of isolation methods and enzymatic reactions

Kit Components

<table>
<thead>
<tr>
<th>Component</th>
<th>Product # 23800 (50 samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding Buffer H</td>
<td>60 mL</td>
</tr>
<tr>
<td>Wash Solution K</td>
<td>27 mL</td>
</tr>
<tr>
<td>Elution Buffer L</td>
<td>15 mL</td>
</tr>
<tr>
<td>Micro 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. These reagents should remain stable for at least 1 year in their unopened containers.

Precautions and Disclaimer

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.

Customer-Supplied Reagents and Equipment

You must have the following in order to use the CleanAll DNA/RNA Clean-up and Concentration Micro Kit:

For All Protocols

- Benchtop microcentrifuge
- Microcentrifuge tubes
- 96 - 100% ethanol

For Endotoxin Removal

- Isopropanol

For RNA Clean-up and Concentration

- β-mercaptoethanol

For RNA Clean-up and Concentration from Phenol/Guanidine-based RNA (Trizol or Tri Reagent) Isolation Methods

- 70% ethanol
**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:

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

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.

Since Norgen’s CleanAll DNA/RNA Clean-up and Concentration Micro Kit uses common solutions for DNA and RNA clean-up, it is important for user to maintain an RNase-free environment if both procedures are to be carried out, either simultaneously or separately.
Flow Chart

Procedure for Purifying DNA/RNA using Norgen’s CleanAll DNA/RNA Clean-up and Concentration Micro Kit

Obtain Nucleic Acid Sample (RNA or DNA) and add Binding Buffer H

Vortex to mix

For RNA Purification:

- Add ethanol
- Bind to column
- SPIN
- Wash two times with Wash Solution K
- Elute RNA with Elution Buffer L
- SPIN
- Purified RNA

For DNA Purification:

- Bind to column
- SPIN
- Wash two times with Wash Solution K
- Elute DNA with Elution Buffer L
- SPIN
- Purified DNA
A. Protocol for DNA Cleanup

Notes Prior to Use

- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the Wash Solution K by adding 54 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated Wash Solution K. This will give a final volume of 81 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- It is recommended that no more than 10 μg of DNA to be used per cleanup.
- The maximum volume of DNA sample that can be processed is 200 μL.

1. Sample Preparation
   a. Adjust the volume of the DNA sample to 100 μL by adding molecular biology-grade water. It is recommended that no more than 10 μg of DNA be used for each column.

      **Note:** If an input volume between 100 and 200 μL is used, adjust the sample volume to 200 μL (maximum allowable) with molecular biology-grade water. In this case, use the volumes indicated in **bold** in the bracket in Steps 1b

   b. Add 500 μL (or 1 mL) of Binding Buffer H to the DNA sample. Mix by vortexing

2. Binding to Column
   a. Assemble a column with one of the provided collection tubes
   b. Apply up to 600 μL of the DNA sample (from Step 1b) onto the column and centrifuge for 1 minute.
   c. Discard the flowthrough. Reassemble the spin column with its collection tube.
   d. If the volume of the DNA sample is greater than 600 μL, repeat Step 2b and 2c until all the remaining DNA sample has passed through the column.

3. Column Wash
   a. Apply 500 μL of Wash Solution K to the column assembly and centrifuge the unit for 2 minutes at 14,000 x g.
   b. Discard the flowthrough and reassemble the unit.
   c. Spin the column for an additional 1 minute at 14,000 x g, in order to completely dry the resin. Discard the collection tube.
   d. Ensure that the column is dry. Spin for an additional minute, if necessary.
   e. Discard the collection tube with the flowthrough.

4. Elution of Clean DNA
   a. Assemble the column with one of the provided 1.7 mL Elution tubes.
   b. Elute DNA with the provided Elution Buffer L according the following table:

<table>
<thead>
<tr>
<th>Elution Volume</th>
<th>10,000 bp or larger</th>
<th>1000 bp to 10,000 bp</th>
<th>Smaller than 1000 bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recommended Elution Volume</td>
<td>100 μL</td>
<td>50 μL</td>
<td>50 μL</td>
</tr>
<tr>
<td>Minimum Elution Volume</td>
<td>50 μL</td>
<td>20 μL</td>
<td>20 μL</td>
</tr>
<tr>
<td>Elution Speed</td>
<td>2 minutes at 200 x g followed by 1 minute at 14,000 x g</td>
<td>2 minutes at 200 x g followed by 1 minute at 14,000 x g</td>
<td>1 minute at 14,000 x g</td>
</tr>
</tbody>
</table>
c. (Optional): An additional elution can be performed by repeating steps 3a and 3b. This elution should be collected into a separate tube to avoid diluting the DNA solution in the first elution.

B. Protocol for DNA Cleanup with Endotoxin Removal

NotesPrior to Use

- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the Wash Solution K by adding 54 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated Wash Solution K. This will give a final volume of 81 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- It is recommended that no more than 10 µg of DNA to be used per cleanup.
- The maximum volume of DNA sample that can be processed is 200 µL.

1. Sample Preparation
   a. Transfer up to 100 µL of DNA into a microcentrifuge tube. Add 500 µL of Binding Buffer H to the DNA and mix well by inversion or vortexing.
   b. Assemble a spin column with a provided collection tube. Add the DNA solution to the top of the column.
   c. Let the column assembly stand for 5 minutes at room temperature.
      
      Note: If dripping occurs into the collection tube during the 5 minute incubation period, just proceed with protocol as written.
   d. After 5 minutes, add 60 µL of isopropanol to the liquid on the column. Close lid and vortex column assembly gently to mix.

2. Binding to Column
   a. Spin the column at 14,000 x g for 1 minute in a microcentrifuge.
   b. Discard the flowthrough and reassemble the spin column with its collection tube.

3. Washing Bound DNA
   a. Apply 500 µL of Wash Solution K to the column assembly and centrifuge the unit for 2 minutes at 14,000 x g.
   b. Discard the flowthrough and reassemble the unit.
   c. Spin the column for an additional 1 minute at 14,000 x g, in order to completely dry the resin. Discard the collection tube.

4. Elution of Clean DNA
   a. Assemble the column with one of the provided 1.7 mL Elution tubes.
   b. Elute DNA with the provided Elution Buffer L according the following table:
<table>
<thead>
<tr>
<th></th>
<th>10,000 bp or larger</th>
<th>1000 bp to 10,000 bp</th>
<th>Smaller than 1000 bp</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recommended Elution Volume</strong></td>
<td>100 μL</td>
<td>50 μL</td>
<td>50 μL</td>
</tr>
<tr>
<td><strong>Minimum Elution Volume</strong></td>
<td>50 μL</td>
<td>20 μL</td>
<td>20 μL</td>
</tr>
<tr>
<td><strong>Elution Speed</strong></td>
<td>2 minutes at 200 x g followed by 1 minute at 14,000 x g</td>
<td>2 minutes at 200 x g followed by 1 minute at 14,000 x g</td>
<td>1 minute at 14,000 x g</td>
</tr>
</tbody>
</table>

**C. Protocol for RNA Clean-up and Concentration from Enzymatic Reactions or Other Non-Phenol/Guanidine-Based RNA Isolation Methods**

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.

**Notes Prior to Use**

- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the Wash Solution K by adding 54 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated Wash Solution K. This will give a final volume of 81 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Prepare an appropriate amount of Binding Buffer H by adding 10 μL of β-mercaptoethanol (provided by the user) to each 1 mL of Binding Buffer H required. β-mercaptoethanol is toxic and should be dispensed in a fume hood.
- It is recommended that no more than 50 μg of RNA to be used per cleanup.
- The maximum volume of RNA sample that can be processed is 200 μL.
- It is important to work quickly during this procedure.
- This kit purifies RNA with minimal amounts of DNA contamination. However, an optional protocol is provided in Appendix A for maximum removal of residual DNA that may affect sensitive downstream applications such quantitative PCR.

**1. Sample Preparation**

a. Adjust the volume of the RNA sample to 100 μL by adding RNase-free or DEPC-treated water. It is recommended that no more than 50 μg of RNA be used for each column.

   **Note:** If an input volume between 100 and 200 μL is used, adjust the sample volume to 200 μL (maximum allowable) with RNase-free or DEPC-treated water. In this case, use the volumes indicated in **bold** in the bracket in Steps 1b and 1c.

b. Add 250 μL (or 500 μL) of Binding Buffer H to the RNA sample. Mix by vortexing

c. Add 200 μL (or 400 μL) of 96 – 100% ethanol (provided by the user) to the mixture from step 1b. Mix by vortexing for 10 seconds.
2. Binding to Column
   a. Assemble a column with one of the provided collection tubes
   b. Apply up to 600 μL of the RNA sample with the ethanol (from Step 1c) onto the column and centrifuge for 1 minute.
   c. Discard the flowthrough. Reassemble the spin column with its collection tube.
   d. If the volume of the RNA sample is greater than 600 μL, repeat Step 2b and 2c until all the remaining RNA sample has passed through the column.

3. Column Wash
   a. Apply 500 μL of Wash Solution K 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. Wash column a second time by adding another 500 μL of Wash Solution K and centrifuging for 2 minutes.
   d. Ensure that the column is dry. Spin for an additional minute, if necessary.
   e. Discard the collection tube with the flowthrough.

4. RNA Elution
   a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
   b. Add 50 μL of Elution Buffer L to the column.
      
      Note: For higher concentrations of RNA, a lower elution volume may be used. A minimum volume of 20 μL is recommended

   c. Centrifuge for 2 minutes at 200 x g (~2,000 RPM), followed by 1 minute 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 RNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat Steps 4b and 4c).

5. Storage of RNA
   The purified RNA sample may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.
D. Protocol for RNA Clean-up and Concentration from Phenol/Guanidine-based RNA (Trizol or Tri Reagent) Isolation Methods

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.

Notes Prior to Use
- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the Wash Solution K by adding 54 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated Wash Solution K. This will give a final volume of 81 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- It is recommended that no more than 50 µg of RNA to be used per cleanup.
- It is important to work quickly during this procedure.

1. Sample Preparation
   a. Isolate RNA using a phenol/guanidine-based reagent such as Trizol or Tri Reagent, according to manufacturer’s instruction. After the separation of the aqueous and organic phases, collect the upper (aqueous) fractions containing the RNA into a new RNase-free microcentrifuge tube (not provided). Note the volume.
   b. Add one volume of 70% ethanol (provided by the user) to the fraction from step 1a. Mix by vortexing for 10 seconds.

2. Binding to Column
   a. Assemble a column with one of the provided collection tubes
   b. Apply up to 600 µL of the RNA mixed with the ethanol (from Step 1b) onto the column and centrifuge for 1 minute.
   c. Discard the flowthrough. Reassemble the spin column with its collection tube.
   d. If the volume of RNA mix is greater than 600 µL, repeat Steps 2b and 2c until all the remaining RNA mix has passed through the column.

3. Column Wash
   a. Apply 500 µL of Wash Solution K 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.
   c. Discard the flowthrough and reassemble the spin column with its collection tube.
   d. Repeat steps 3a and 3b to wash the column a second time.
   e. Wash column a third time by adding another 500 µL of Wash Solution K and centrifuging for 2 minutes.
   f. Ensure that the column is dry. Spin for an additional minute, if necessary.
   g. Discard the collection tube with the flowthrough.

4. RNA Elution
   a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
   b. Add 50 µL of Elution Buffer L to the column.
Note: For higher concentrations of RNA, a lower elution volume may be used. A minimum volume of 20 μL is recommended.

c. Centrifuge for 2 minutes at 200 x g (~2,000 RPM), followed by 1 minute 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 RNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat Steps 4b and 4c).

5. Storage of RNA
The purified RNA sample 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: Optional DNA Removal in Solution Followed by RNA Clean-up and Concentration

Norgen’s CleanAll DNA/RNA Clean-up and Concentration Micro Kit purifies RNA with minimal amounts of DNA contamination. An optional protocol is provided below for maximum removal of residual DNA that may affect sensitive downstream applications such as quantitative PCR. It is recommended that an RNase-free DNase I be used.

1. Adjust the volume of the RNA sample to be treated to 80 μL with RNase-free water.

2. Prepare a working stock of 0.5 Kunitz unit/μL RNase-free DNase I solution according to the manufacturer’s instructions. Alternatively, dissolve or dilute stock DNase I in a reaction buffer (40 mM Tris pH 7.0, 10 mM MgCl₂ and 3 mM CaCl₂, made RNase-free) to give a final concentration of 0.5 Kunitz unit/μL.

3. Add 20 μL of 0.5 Kunitz unit/μL DNase I to the RNA sample.

4. Incubate at 25 to 30°C for 15 minutes.

5. Proceed directly to Protocol A “Protocol for RNA Clean-up and Concentration from Enzymatic Reactions or Other Sample Preparation Methods”.
## Troubleshooting Guide

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution and Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor DNA recovery</td>
<td>Binding of DNA to the column was inefficient</td>
<td>Binding of the DNA is dependent on both pH and salt concentration. Ensure that an appropriate amount of Binding Buffer H was used for the volume of the DNA Input.</td>
</tr>
<tr>
<td>Poor DNA recovery</td>
<td>The appropriate amount of ethanol was not added to the Wash Concentrate</td>
<td>The Wash Solution K has been specifically designed to contain the appropriate amount of components. Ensure that the Wash Solution K was prepared using the correct amount of ethanol.</td>
</tr>
<tr>
<td>Poor DNA recovery</td>
<td>Binding Buffer H was not completely removed in the wash step.</td>
<td>Traces of salt left on the column from the binding step may interfere with the elution of the DNA. Ensure that the column is washed with the Wash Solution K.</td>
</tr>
<tr>
<td>Poor DNA recovery</td>
<td>Proper Elution Buffer was not used</td>
<td>The provided Elution Buffer L has been optimized for high elution recoveries. If water or TE buffer is used instead, ensure the pH is around 8.</td>
</tr>
<tr>
<td>Poor DNA recovery</td>
<td>Elution Buffer L was not placed directly onto the resin</td>
<td>It is important that the Elution Buffer L be placed directly onto the resin, as this helps to increase recovery by ensuring an even passing of the buffer through the resin. Do not pipette the Elution Buffer L onto the side of the column.</td>
</tr>
<tr>
<td>DNA does not perform well in downstream applications</td>
<td>Insufficient washing of resin with bound DNA</td>
<td>Traces of salt from the binding step may remain in the sample if the column is not properly washed with the Wash Solution K. Ensure that the column is spun for 2 minutes during the washing step. Salt may interfere with downstream applications, and thus must be washed from the column.</td>
</tr>
<tr>
<td>Endotoxin levels in the eluted DNA are slightly higher than 0.1 EU/µg DNA</td>
<td>Proper Elution Buffer was not used</td>
<td>The provided Elution Buffer L has been optimized for endotoxin-free recoveries. If endotoxin-free water is used for the elution, ensure that the pH is between 7 and 8.</td>
</tr>
<tr>
<td>Endotoxin levels in the eluted DNA are slightly higher than 0.1 EU/µg DNA</td>
<td>A different Elution Buffer was used</td>
<td>The provided Elution Buffer L has been optimized for endotoxin-free recoveries. The endotoxin-free properties of the eluted DNA will be compromised if another elution buffer is used. If a different Elution buffer other than the one provided is used, the buffer should also be checked for endotoxin levels.</td>
</tr>
<tr>
<td>Endotoxin levels in the eluted DNA are slightly higher than 0.1 EU/µg DNA</td>
<td>The endotoxin levels of the input were extremely high</td>
<td>If the initial input DNA had extremely high endotoxin levels, the levels may not be completely reduced to 0.1 EU/µg of DNA or less. In this case, the eluted DNA could be applied to a second column and the procedure repeated in order to further reduce the endotoxin levels.</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>Poor RNA Recovery</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 <strong>Elution Buffer L</strong> supplied with this kit be used for maximum RNA recovery.</td>
</tr>
<tr>
<td></td>
<td>Ethanol was not added to the lysate</td>
<td>Ensure that the appropriate amount of ethanol is added to the lysate before binding to the column.</td>
</tr>
<tr>
<td></td>
<td>Ethanol was not added to the Wash Solution K</td>
<td>Ensure that 54 mL of 96-100% ethanol is added to the supplied <strong>Wash Solution K</strong> prior to use.</td>
</tr>
<tr>
<td>Clogged Column</td>
<td>High amounts of RNA as input</td>
<td>Ensure that no more than 50 μg of RNA are used as input</td>
</tr>
<tr>
<td></td>
<td>High amounts of genomic DNA present in sample</td>
<td>The lysate may be passed through a 25 gauge needle attached to a syringe 5-10 times in order to shear the genomic DNA prior to loading onto the column.</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>RNA is Degraded</td>
<td>RNase contamination</td>
<td>RNases may be introduced during the use of the kit. Proper procedures are followed when working with RNA. Please refer to “<strong>Working with RNA</strong>” 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.</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>Problem</td>
<td>Possible Cause</td>
<td>Solution and Explanation</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>-----------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>RNA does not perform well in downstream applications</td>
<td>RNA was not washed twice with the provided <strong>Wash Solution K</strong></td>
<td>Traces of salt from the binding step may remain in the sample if the column is not washed twice times with <strong>Wash Solution K</strong>. Salt may interfere with downstream applications, and thus must be washed from the column.</td>
</tr>
<tr>
<td></td>
<td>Ethanol carryover</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>DNA or Genomic DNA contamination</td>
<td>Large amounts of starting material used</td>
<td>Perform RNAse-free DNase digestion on the RNA sample before clean-up according to the protocol provided in <strong>Appendix A</strong>.</td>
</tr>
</tbody>
</table>

**Related Products**

<table>
<thead>
<tr>
<th>Product</th>
<th>Product #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total RNA Purification Kit</td>
<td>17200</td>
</tr>
<tr>
<td>Cytoplasmic &amp; Nuclear RNA Purification Kit</td>
<td>21000</td>
</tr>
<tr>
<td>Leukocyte RNA Purification Kit</td>
<td>21200</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.

Norgen’s purification technology is patented and/or patent pending. See www.norgenbiotek.com/patents

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