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RNase-Free DNase I Kit — Product Insert

Product # 25720

Norgen's RNA purification kits isolate total RNA with minimal amounts of genomic DNA contamination. However, for some sensitive downstream applications, it may be desirable to remove all traces of residual DNA. Norgen's RNase-free DNAse I Kit, with Enzyme Incubation Buffer, can be used for optional on-column DNase digestion with any of Norgen's RNA purification kits. Alternatively, after isolating total RNA using one of Norgen's RNA purification kits, the RNA elution can be treated with this DNase I. The RNA can then be purified from the DNase using Norgen's RNA Clean-Up and Concentration Kit (Cat# 23600), and the RNA can then be used in downstream applications. The amount of DNase I provided with this product is enough to perform 200 reactions.

Kit Components

Component	Product # 25720 (200 reactions)
DNase I	4 x 1 vial
Enzyme Incubation Buffer	4 x 6 mL
Product Insert	1

Storage Conditions and Product Stability

The Enzyme Incubation Buffer should be stored at room temperature. The DNase I should be stored at -20°C. 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*.

Procedures

A. Protocol for On-Column DNA Removal using Norgen's RNA Purification Kits

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. Please check your microcentrifuge specifications to ensure proper speed. All centrifugation steps are performed at room temperature.
- This protocol is written to be incorporated as the optional on-column DNase digest step in Norgen's RNA purification kits.
- Standard DNase buffers are not compatible with on-column DNase digestion and may affect the binding of the RNA to the column. Use of other buffers may reduce RNA yield and integrity.

1. For every on-column reaction to be performed, prepare a mix of 15 µL of **DNase I** and 100 µL of **Enzyme Incubation Buffer**. Mix gently by inverting the tube a few times. **DO NOT VORTEX**.

2. Perform the appropriate RNA isolation procedure for your starting material up to and including the "**Binding to Column**" step.
3. Apply 400 µL of **Wash Solution** (provided with purification kit) to the column and centrifuge for 2 minutes. Discard the flowthrough. Reassemble the spin column with its collection tube.
4. Apply the 115 µL of **DNase I + Enzyme Incubation Buffer** to the column and centrifuge at 14,000 x g (~14,000 RPM) for 1 minute.

   Note: Ensure that the entire 115 µL of **DNase I + Enzyme Incubation Buffer** mix passes through the column. If needed, spin at 14,000 x g (~14,000 RPM) for an additional minute.

5. After the centrifugation in Step 4, pipette the flowthrough that is present in the collection tube back onto the top of the column.

   Note: Ensure Step 5 is performed in order to ensure maximum DNase activity and to obtain maximum yields of RNA, in particular for small RNA species.

6. Incubate the column assembly at 25 - 30°C for 15 minutes.
7. Without any further centrifugation, proceed directly to the **second wash step** of the "**Column Wash**" section of relevant purification kit protocol.

B. Protocol for DNA Removal in Solution Followed by RNA Clean-Up

Notes prior to use:

- This protocol describes how to digest DNA in RNA solutions prior to cleanup using Norgen's RNA Clean-Up and Concentration Kit (Cat# 23600).
- The input for this procedure is an RNA elution from any of Norgen's RNA purification kits which may be contaminated with trace amounts of DNA.

1. In a microcentrifuge tube mix together 10 µL of **Enzyme Incubation Buffer**, 2.5 µL of **DNase I**, and up to 87.5 µL of RNA solution (contaminated with DNA). Bring the volume to 100 µL using RNase-free water.
2. Incubate at room temperature (20-25°C) for 10 minutes.
3. Purify the RNA from the DNase using Norgen's RNA Clean-Up and Concentration Kit (Cat# 23600).

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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