

## RNase-Free DNase I Kit

Product # 25710, 25720

## Product Insert

### Kit Components

Component	Product # 25710 (50 reactions)	Product # 25720 (200 reactions)
DNase I	1 vial / 1,600 units	4 vials (1,600 units/vial)
Buffer DR	1 mL	4 x 1 mL
Enzyme Incubation Buffer	6 mL	4 x 6 mL
Product Insert	1	1

### Storage Conditions and Product Stability

The DNase I provided is in lyophilized form. It is stable for at least 3 months if stored at room temperature. However, it is recommended to store the DNase I vial at 2 – 8°C (or below) upon receipt to maintain stability beyond 3 months. Buffer DR and Enzyme Incubation Buffer can be stored at room temperature. After reconstitution with Buffer DR (see below), the DNase I should be stored at -20°C. All reagents should remain stable for at least 1 year in their unopened containers at the appropriate storage temperature.

### 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.norgenbiotech.com](http://www.norgenbiotech.com).

## Procedures

### Notes prior to use:

- **Product # 25710:** Reconstitute the DNase I by transferring 800 µL of Buffer DR into the vial of DNase I. Pipette up and down gently to mix. **Do not vortex.** If needed, aliquot the DNase I into smaller portions and store the unused portions at -20°C.
- **Product # 25720:** Reconstitute each vial of DNase I by transferring 800 µL of Buffer DR into one vial of DNase I. Pipette up and down gently to mix. **Do not vortex.** If needed, aliquot the DNase I into smaller portions and store the unused portions at -20°C.
- 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.

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

1. For every on-column reaction to be performed, prepare a mix of 15  $\mu\text{L}$  of **DNase I** and 100  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  of **Enzyme Incubation Buffer**, 2.5  $\mu\text{L}$  of **DNase I**, and up to 87.5  $\mu\text{L}$  of RNA solution (contaminated with DNA). Bring the volume to 100  $\mu\text{L}$  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](http://www.norgenbiotek.com)) or through email at [techsupport@norgenbiotek.com](mailto:techsupport@norgenbiotek.com).