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DNase Max® Handbook

For the removal of genomic DNA contamination in RNA preparations

Sample to Insight

QIAGEN

Contents

Kit Contents

DNase Max Kit	**(50)**
Catalog no.	**15200-50**
Number of preps	**50**
DNase I Enzyme	55 µl
DNase Removal Resin	550 µl
10x DNase Buffer	550 µl
RNase-Free water	2 x 1 ml
Quick Start Protocol	1

Storage

The DNase Max Kit reagents and components are stable room temperature (15–25°C) for up to 6 months and for two years at 2–8°C without loss of activity.

Intended Use

The DNase Max Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

All due care and attention should be exercised in the handling of the product. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of DNase Max Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

When analyzing RNA, it is important to get the cleanest sample possible. The DNase Max Kit removes DNA contamination from RNA preparations, improving the quality and clarity of downstream results. The basis of the DNase Max Kit is a highly purified DNase I enzyme formulated for long-term stability at room temperature using a proprietary stabilization solution. The kit enables removal of up to 30 µg DNA in 20 minutes, using 10 units (1 µl) of enzyme.

The DNase Max Kit is stable at room temperature (15–25°C) for up to 6 months or at 2–8°C for 2 years with no loss of activity. Room temperature storage eliminates the need to aliquot and freeze stocks of DNase I enzyme and removes concerns about decreased enzyme activity due to freeze–thaw cycles.

Principle and procedure

Starting with a previously processed sample, DNase I enzymes break down unwanted DNA. Then, a highly specific, novel resin binds to the enzyme and divalent cations produced by the reaction. Neither heat nor EDTA is needed to inactivate the DNase. RNA processed with the kit is immediately ready for use in downstream applications.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Microcentrifuge (13,000 x *g*)
- Pipettes (1.5–1000 µl)
- Optional: Vortex-Genie® 2 Vortex
- Optional: Vortex Adapter for 24 (1.5–2.0 ml) tubes (cat. no. 13000-V1-24)

Protocol: Removal of Genomic DNA

Important points before starting

- Do not vortex the DNase I. It will denature the enzyme and decrease its activity.
- Just before use, resuspend the DNase Removal Resin by inverting or vortexing until slurry is homogeneous.

DNase reaction

1. Mix 1 µl DNase I enzyme (10 units) and enough 10x DNase Buffer to achieve a final concentration of 1x DNase Buffer in the digestion reaction.

   Examples: For 50 µl digestion reactions, use 1 µl of DNase I enzyme and 5 µl of 10x DNase Buffer. For 100 µl reactions, use 1 µl DNase I enzyme and 10 µl of 10x DNase Enzyme Buffer.
2. Bring the reaction to final volume using RNase-Free Water (provided). Mix by pipetting up and down.
3. Incubate at 37°C for 20 min.

DNase removal

4. Add 5 µl homogenous DNase Removal Resin per 10 units of DNase I for a 50 µl reaction or 10 µl DNase Removal Resin for every 100 µl reaction; whichever is greater.
5. Incubate for 10 min at room temperature. Invert or flick to resuspend every 1–2 min or place the tubes on a Vortex Adapter attached to a Vortex Genie 2 and set the vortex between speed 5–6 to agitate the resin and promote binding of the DNase.

   Note: The solution should agitate without splashing.
6. Centrifuge at 13,000 x *g* for 1 min to pellet the resin.
7. Transfer the supernatant to a new tube, taking care not to transfer any of the resin. The RNA is now ready to use for RT-PCR and further analysis.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
RNA	
a) RNA does not work in RT-PCR	Check the RNA on an agarose gel before DNase digest to ensure that you are working with high-quality RNA. Use a 1% TAE agarose gel to check 10 µl of RNA at a concentration range between 10–100 ng/µl. Ensure DNase Removal Resin was not transferred with the final RNA. You may need to leave 1–2 µl of sample behind. If you disturb the resin bed when removing the DNase digested RNA, re-pellet the resin again to pack down before removing the RNA.
b) RNA still contains residual DNA	When performing PCR or qPCR on DNase treated RNA to determine if DNA is still present, make sure to include a no template control reaction (water alone) to rule out contamination of DNA from another source. Prior to PCR analysis, perform an agarose gel of the RNA to determine the level of DNA contamination of your RNA. If more than 30 µg of DNA is present, it may be necessary to use more enzyme or to allow the digest to continue longer than 20 minutes. Quantification with PicoGreen® may be used to determine the concentration of DNA present in the sample, if necessary. If the 20-minute digest at 37°C does not completely remove all traces of DNA, the digest may be extended to 30 minutes. The DNase enzyme will digest up to 30 µg of DNA with 10 units of enzyme in 20 minutes. Make sure that the DNase I enzyme was not vortexed as this will decrease the activity of the enzyme. Vortexing during the removal step in the presence of the resin is fine. Make sure that the 10x DNase Buffer was added to the reaction at a final concentration of 1x. The highest activity of DNase is achieved using the buffer supplied with this enzyme.

Ordering Information

Product	Contents	Cat. no.
DNase Max Kit (50)	For 50 preps: For the removal of genomic DNA contamination in RNA preparations	15200-50
Related products		
Vortex Adapter for 24 (1.5–2.0 ml) tubes	For vortexing 1.7, 2, 5, 15 and 50 ml tubes using the Vortex-Genie 2 Vortex.	13000-V1-24

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Note

Handbook Revision History

Date	Changes
08/2019	Correction of quantity of digestion reactions in the protocol from 10 µl to 50 µl.
02/2018	Correction of mistake in kit name in “Introduction”, page 5.
10/2017	Initial release.