ambion®
by life technologies™

DNA-*free*™ Kit
DNase Treatment and Removal Reagents

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Product description

The Ambion® DNA-*free*™ DNase Treatment and Removal Reagents are designed to remove contaminating DNA from RNA preparations, and to subsequently remove the DNase and divalent cations from the sample. The recombinant DNase I (rDNase I) provided in the kit is overexpressed in an animal-free system, and is then extensively purified in a bovine-free process and tested. It is guaranteed to lack any contaminating RNase activity. The kit also includes an optimized DNase reaction buffer. Using the DNA-*free*™ reagents, contaminating DNA is digested to levels below the limit of detection by routine PCR. The DNase is then removed rapidly and easily using a novel method which does not require phenol/chloroform extraction, alcohol precipitation, heating, or the addition of EDTA (see Figure 1). RNA treated with DNA-*free*™ reagents is suitable for endpoint or real-time RT-PCR (Figure 2), microarray analysis, RPAs, Northerns, and all other RNA analysis methods.

In addition to removing the DNase enzyme, DNase Inactivation Reagent also removes divalent cations, such as magnesium and calcium, which can catalyze RNA degradation when RNA is heated with the sample (Figure 3).

Figure 1 Inactivation of rDNase I by DNase Inactivation Reagent. The indicated amount of rDNase I was added to 50 µL of 1X DNase I Buffer. The sample in lane 3 was treated with 5 µL of DNase Inactivation Reagent. Next, genomic Lambda DNA (1 µg) was added to each tube and incubated for 10 min at 37°C to test for residual DNase activity. Reaction were analyzed on an ethidium bromide-stained agarose gel. Note that treatment with the DNase Inactivation Reagent inactivated the rDNase I (Lane 3), preventing digestion of the DNA.

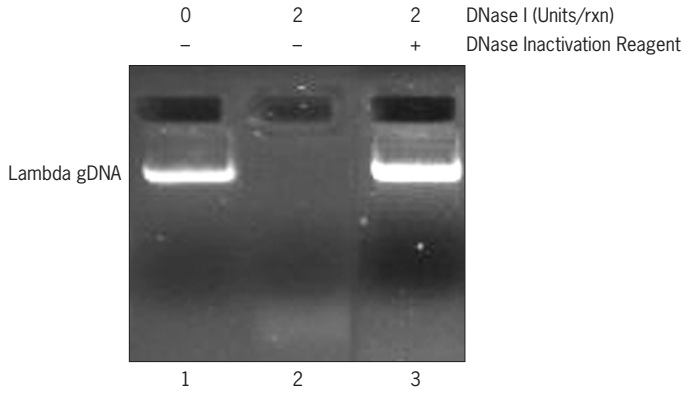

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Figure 2 **Total RNA treated with DNA-*free*™ reagents is compatible with real-time RT-PCR**. 10-fold serial dilutions from 100 ng to 10 pg of HeLa-S3 total RNA was treated with DNA-*free*™ reagents and then the DNase was either removed by following the procedure for routine DNA removal (A1–A5) or was phenol/chloroform extracted to remove the DNase (B1–B5). The two samples were then tested in one step real-time RT-PCR for detection of the human CDC gene with TaqMan® detection. (Instrument: ABI Prism® 7000 Sequence Detection System.)

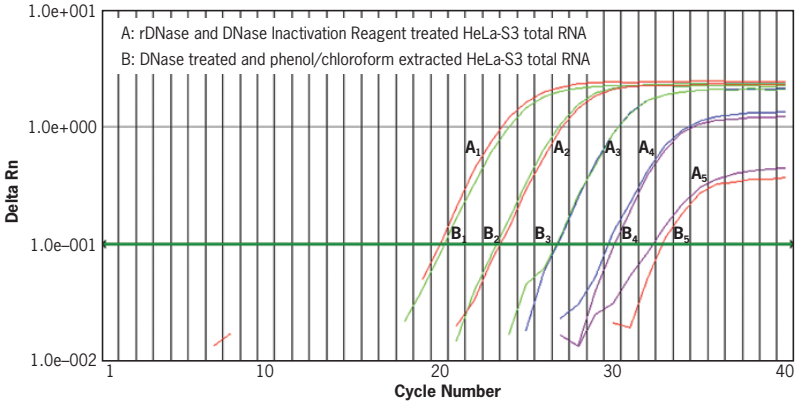
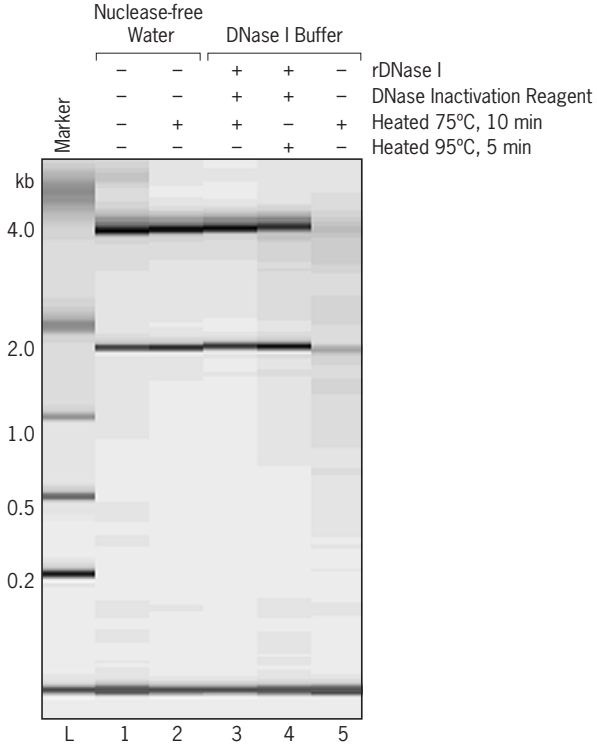


Figure 3 **Removal of divalent cations by DNase Inactivation Reagent**. HeLa-S3 total RNA (100 ng) in 50 µL 1X DNase I Buffer or in nuclease-free water was treated with components from the DNA-*free*™ Kit as indicated. Samples were heated for 10 min at 75°C to determine if divalent cations from the Dnase I Buffer remained in solution, and degraded the RNA. 1 µL of each sample was analyzed on an RNA LabChip® using the Agilent 2100 Bioanalyzer™ Instrument. Note that RNA was degraded in the sample that contained DNase I Buffer, but was not treated with the DNase Inactivation Reagent; this degradation is due to the presence of divalent ions that induce heat-mediated RNA cleavage.



Procedure overview

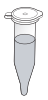
For the detailed procedure, see section "DNA-free™ Kit procedure" on page 4.

Add DNase Digestion Reagents

1. Add 0.1 volume 10X DNase I Buffer and 1 µL rDNase I to the RNA, and mix gently. (page 4)

Incubate

2. Incubate at 37°C for 20–30 min. (page 5)

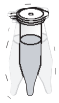


Add DNase Inactivation Reagent

3. Add resuspended DNase Inactivation Reagent (typically 0.1 volume) and mix well. (page 5)

Incubate and mix

4. Incubate 2 min at room temperature, mixing occasionally. (page 5)



Centrifuge and transfer RNA

5. Centrifuge at 10,000 × g for 1.5 min and transfer the RNA to a fresh tube. (page 5)

How much RNA can be treated with the DNA-*free*™ Kit?

This protocol is designed to remove trace to moderate amounts of contaminating DNA (up to 50 µg DNA/mL RNA) from purified RNA to a level that is mathematically insignificant by RT-PCR. No RNA isolation method can extract RNA that is completely free from DNA contamination; in fact, RNA isolated from some tissues, such as spleen, kidney, or thymus, often contain relatively high levels of DNA. Other potential sources of DNA contamination include carryover of the interface during organic extractions, and overloaded glass-fiber filters during RNA purification.

DNA-*free*™ Kit components and storage

Reagents are provided for 50 DNA-*free*™ Kit treatments (up to 100 µL each).

Amount	Component	Storage
120 µL	rDNase I (2 Units/µL)	–20°C
600 µL	10X DNase I Buffer†	
600 µL	DNase Inactivation Reagent	
1.75 mL	Nuclease-free Water	any temperature‡

† 10X: 100 mM tris-HCl pH 7.5, 25 mM MgCl2, 5 mM CaCl2

‡ Store Nuclease-free Water at –20°C, 4°C or room temperature.

Store the DNA-*free*™ Kit at –20°C in a non-frost-free freezer for long-term storage. For convenience, the 10X DNase I Buffer and the DNase Inactivation Reagent can be stored at 4°C for up to 1 week.

Note: If additional DNase is required, the rDNase I (recombinant DNase I) included with the DNA-*free*™ Kit can be purchased separately from Life Technologies (Cat no. AM2235, 1000 U). We do NOT recommend using wild-type DNase I derived from bovine pancreas with the DNA-*free*™ Kit.

DNA-*free*™ Kit procedure

Procedure notes

- We recommend conducting reactions in 0.5 mL tubes to facilitate removal of the supernatant after treatment with the DNase Inactivation Reagent.
- DNA-*free*™ reactions can be conducted in 96-well plates. We recommend using V-bottom plates because their shape makes it easier to remove the RNA from the pelleted DNase Inactivation Reagent at the end of the procedure.
- The recommended reaction size is 10–100 µL. A typical reaction is 50 µL.

Procedure

1. Add 0.1 volume 10X DNase I Buffer and 1 µL rDNase I to the RNA, and mix gently.

   There are separate DNase digestion conditions depending on the amount of contaminating DNA and the nucleic acid concentration of the sample.

   - **Routine DNase treatment**: ≤ 200 µg nucleic acid per mL
   - **Rigorous DNase treatment**: > 200 µg nucleic acid per mL or RNA that is severely contaminated with DNA (i.e. >2 µg DNA/50 µL)

   Routine DNase treatment: Use 1 µL rDNase I (2 U) for up to 10 µg of RNA in a 50 µL reaction. These reaction conditions will remove up to 2 µg of genomic DNA from total RNA in a 50 µL reaction volume.

   Rigorous DNase treatment: If the RNA contains more than 200 µg of nucleic acid per mL, dilute the sample to 10 µg nucleic acid/50 µL before adding the DNase I Buffer and rDNase I. It is also helpful to add only half of the rDNase I to the reaction initially, incubate for 30 min, then add the remainder of the enzyme and incubate for another 30 min.

If the sample cannot be diluted, simply increase the amount of rDNase I to 2–3 µL (4–6 U). It may be possible to successfully remove contaminating DNA from samples containing up to 500 µg/mL nucleic acid in a 10–100 µL DNA-*free*™ Kit reaction. However, the efficacy of treating highly concentrated nucleic acid samples depends on the absolute level of DNA contamination, and residual DNA may or may not be detectable by PCR after 35–40 cycles.

2. Incubate at 37°C for 20–30 min.

   If only half of the rDNase I was added in step 1, incubate for 30 min, then add the rest of the rDNase I and incubate for 30 min more.

3. Add resuspended DNase Inactivation Reagent (typically 0.1 volume) and mix well.

   Always resuspend the DNase Inactivation Reagent by flicking or vortexing the tube before dispensing it.

   - **For routine DNase treatment**: use 2 µL or 0.1 volume DNase Inactivation Reagent, whichever is greater. For example, if the RNA volume is 50 µL, and 1 µL of rDNase I was used in the step 1, add 5 µL of DNase Inactivation Reagent.
   - **For rigorous DNase treatments**: where 2–3 µL of rDNase I was used, add 0.2 volumes of DNase Inactivation Reagent.

   IMPORTANT! Always use at least 2 µL of DNase Inactivation Reagent, even if it is more than 0.1 volume.

   Note: The DNase Inactivation Reagent may become difficult to pipette after multiple uses due to depletion of fluid from the interstitial spaces. If this happens, add a volume of Nuclease-free Water (supplied with the kit) equal to approximately 20–25% of the bed volume of the remaining DNase Inactivation Reagent, and vortex thoroughly to recreate a pipettable slurry.

4. Incubate 2 min at room temperature, mixing occasionally.

   It is important to mix the contents of the tube 2–3 times during the incubation period to redisperse the DNase Inactivation Reagent.

5. Centrifuge at 10,000 × g for 1.5 min and transfer the RNA to a fresh tube.

   - For 96-well plates, centrifuge at 2000 × g for 5 min.

   This centrifugation step pellets the DNase Inactivation Reagent. After centrifuging, carefully transfer the supernatant, which contains the RNA, into a fresh tube. Avoid introducing the DNase Inactivation Reagent into solutions that may be used for downstream enzymatic reactions, because it can sequester divalent cations and change the buffer conditions.

Troubleshooting

Observation	Possible cause	Recommended action
DNA contamination remains after the initial treatment.	According to a recent publication†, RNA samples from tissue or cells purified with column-based methods may contain significant DNA contamination—as much as 20–50% of the prep. The study found that the nucleic acid content of samples varied widely from consisting of nearly pure RNA to containing mostly DNA. Samples with DNA contamination that remains after completing the DNA-*free*™ Kit procedure may benefit from a second round of rDNase I treatment.	For this second treatment, add 0.15 volumes 10× DNase I Buffer and 1–2 µL rDNase I to the sample, and incubate at 37°C for 20–30 min. After the DNase digestion, follow the standard inactivation procedure starting at step 3. For RT-PCR with double DNA-*free*™ -treated RNA samples, we recommend limiting the volume of treated RNA to 20% of the final RT-PCR volume.
No RT-PCR product is detectable from RNA treated with DNA-*free*™ reagents.	DNase Inactivation Reagent could inhibit RT-PCR.	In step 5 on page 5, remove the RNA solution from the pelleted DNase Inactivation Reagent carefully to avoid transferring it to the tube of RNA. You may have to leave a small amount of RNA behind to accomplish this. If you accidentally touch the pellet while removing the RNA, recentrifuge to pack the DNase Inactivation Reagent.
	DNA-*free*™ -treated RNA should comprise only ~20% of an RT-PCR reaction volume.	For RT-PCR, we recommend that DNA-*free*™ Kit treated RNA makes up ~20%, and no more than 40%, of the final RT-PCR volume. Otherwise, components from the DNase I Buffer and the DNase Inactivation Reagent could interfere with the reaction. If necessary, RT-PCR volumes can be increased to 50 µL or more to accommodate your RNA without exceeding the 20–40% limit.
RNA is degraded upon heating to >60°C.	RNA samples that contain divalent cations, such as magnesium or calcium, will degrade when heated to temperatures above 60°C.	To ensure that divalent cations are removed in step 4 on page 5, redisperse the DNase Inactivation Reagent by mixing the reaction 2–3 times during the incubation period.

† Bustin SA (2002) Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems. *J Mol Endocrinol* 29: 23–39

Quality control

Functional testing

The activity of the rDNase I is tested functionally in a unit activity assay. One unit is defined as the amount of enzyme required to completely degrade 1 µg DNA in 10 min at 37° C. Results are analyzed by agarose gel electrophoresis. The DNase Inactivation Reagent is tested for its ability to remove both rDNase I and DNase I Buffer components. Results are analyzed by agarose gel electrophoresis and the Agilent 2100 bioanalyzer, respectively.

Nuclease testing

Relevant kit components are tested in the following nuclease assays:

RNase activity

A sample is incubated with labeled RNA and analyzed by PAGE.

Nonspecific endonuclease activity

A sample is incubated with supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.

Exonuclease activity

A sample is incubated with labeled double-stranded DNA, followed by PAGE analysis.

Protease testing

A sample is incubated with protease substrate and analyzed by fluorescence.

Appendix A Safety

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Documentation and support

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Limited product warranty

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Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA
Phone +1 760 603 7200 | Toll Free in USA 800 955 6288
For support visit lifetechnologies.com/support or email techsupport@lifetech.com

lifetechnologies.com

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