DNA-*free*™ Kit

DNase treatment and removal reagents

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Invitrogen™ 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 on page 4).

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 2 on page 4).

RNA treated with DNA-*free*™ reagents is suitable for endpoint or real-time RT-PCR (Figure 3 on page 5), microarray analysis, RPAs, Northerns, and all other RNA analysis methods.

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).

Component	Amount	Storage
rDNase I (2 Units/µL)	120 µL	–20°C
10X DNase I Buffer [1]	600 µL	
DNase Inactivation Reagent	600 µL	
Nuclease-free Water	1.75 mL	–20°C, 4°C, or room temperature.

[1] 10X: 100 mM tris-HCl pH 7.5, 25 mM $MgCl_2$, 5 mM $CaCl_2$

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.

Procedure overview

For the detailed procedure, see section ""Perform routine DNase treatment" on page 3".

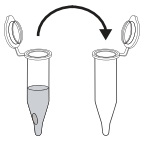
Add DNAase digestion reagents

Incubate sample

Add DNase Inactivation Reagent

Incubate sample with occasional mixing

Centrifuge sample



Transfer sample to a clean tube

DNA-*free*™ kit procedure

Procedure notes

- A typical reaction volume is 50 µL, but can vary in size from 10–100 µL.
- Perform reactions in 0.5 mL tubes to simplify removal of the supernatant after treatment with the DNase Inactivation Reagent.
- Reactions can also be conducted in 96-well plates. Plates with V-bottom wells are recommended because their shape makes it easier to remove the RNA from the pelleted DNase Inactivation Reagent at the end of the procedure.
- There are two methods for DNase treatment depending on the amount of contaminating DNA and the nucleic acid concentration of the sample.
  - **Routine DNase treatment:** Sample contains ≤200 µg nucleic acid per mL. 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 (see "Perform routine DNase treatment" on page 3).
  - **Rigorous DNase treatment:** Sample contains >200 µg nucleic acid per mL. Dilute the sample to prior to 2 µg of nucleic acid per mL before adding DNase I Buffer and rDNase I. In addition, rDNase I treatment can be divided into two steps (see "Perform rigorous DNase treatment" on page 3).
    If the sample cannot be diluted, simply increase the amount of rDNase I to 2–3 µL (4–6 U). Increasing the amount of enzyme may successfully remove contaminating DNA from samples containing up to 500 µg/mL nucleic acid in a 10–100 µL 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.

Perform routine DNase treatment

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

2. Incubate at 37°C for 20–30 minutes.

3. Resuspend the DNase Inactivation Reagent by flicking or vortexing the tube before use.

   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. Add resuspended DNase Inactivation Reagent (2 µL or 0.1 volume, whichever is greater), then mix well.

   For example, if the RNA volume is 50 µL, and 1 µL of rDNase I was used in the previous step, add 5 µL of DNase Inactivation Reagent.

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

5. Incubate 2 minutes at room temperature, mixing occasionally.

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

6. Centrifuge the samples, then transfer the supernatant containing the RNA to a fresh tube.
   - Centrifuge microcentrifuge tubes at 10,000 × *g* for 1.5 minutes.
   - Centrifuge 96-well plates at 2000 × *g* for 5 minutes.

   Do not disturb the DNase Inactivation Reagent pellet. 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.

Perform rigorous DNase treatment

1. Dilute the sample to 10 µg nucleic acid/50 µL of total sample if possible.

2. Add 0.1 volume 10X DNase I Buffer, then mix gently.

3. Add rDNase I to the RNA, then mix gently.
   - Use 1 µL rDNase I for diluted samples.
   - Use 2–3 µL (4–6 U) rDNase I for samples that cannot be diluted.

   (*Optional*) DNase treatment can be enhanced by performing a two-step incubation. To perform two-step incubation, add half the amount of DNase I to the sample.

4. Incubate at 37°C for 20–30 minutes.

   (*Optional*) If performing two-step incubation, incubate at 37°C for 30 minutes, then add the remaining half of rDNase I and incubate for another 30 minutes.

5. Resuspend the DNase Inactivation Reagent by flicking or vortexing the tube before use.

   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.

6. Add resuspended DNase Inactivation Reagent, then mix well.
   - Use 0.1 volumes if samples were treated with 1 µL rDNase I.
   - Use 0.2 volumes if samples were treated with 2–3 µL rDNase I.

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

7. Incubate 2 minutes at room temperature, mixing occasionally.

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

8. Centrifuge the samples, then transfer the supernatant containing the RNA to a fresh tube.
   - Centrifuge microcentrifuge tubes at 10,000 × *g* for 1.5 minutes.
   - Centrifuge 96-well plates at 2000 × *g* for 5 minutes.

   Do not disturb the DNase Inactivation Reagent pellet. 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.

Results with the TURBO DNA-*free*™ Kit

Inactivation of rDNase I by DNase inactivation reagent

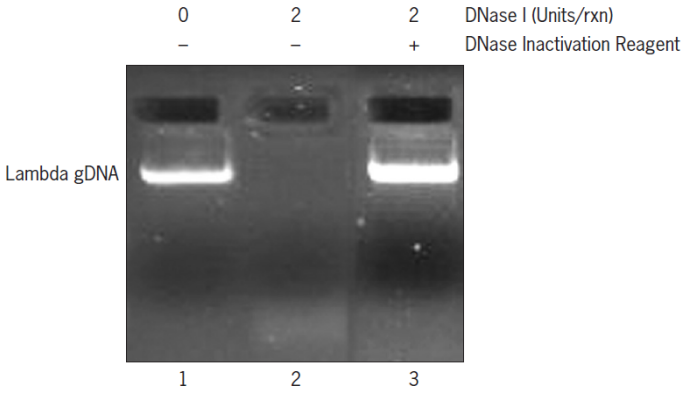


Figure 1 The indicated amount of rDNase I was added to 50 µL of 1X DNase I buffer. The sample in lane 3 was treated with 5 µL of DNase inactivation reagent. Next, genomic lambda DNA (1 µg) was added to each tube and incubated for 10 min at 37°C to test for residual DNase activity. Reaction were analyzed 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.

Removal of divalent cations by DNase inactivation reagent

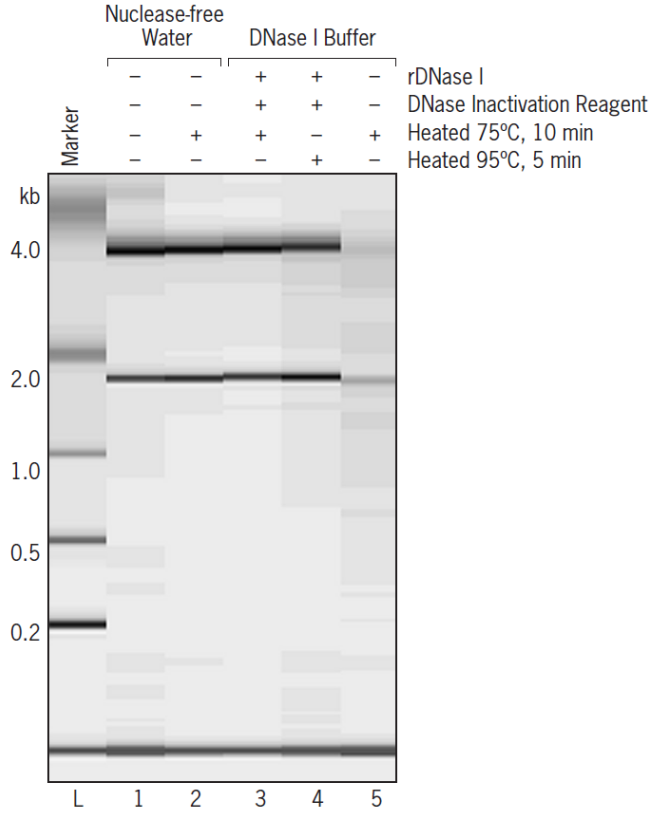


Figure 2 HeLa-S3 total RNA (100 ng) in 50 µL 1× DNase I buffer or in nuclease-free water was treated with components from the DNA-*free*™ Kit as indicated. Samples were heated for 10 min at 75°C to determine if divalent cations from the Dnase I buffer remained in solution, and degraded the RNA. 1 µL 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.

Compatability of treated RNA with real-time RT-PCR

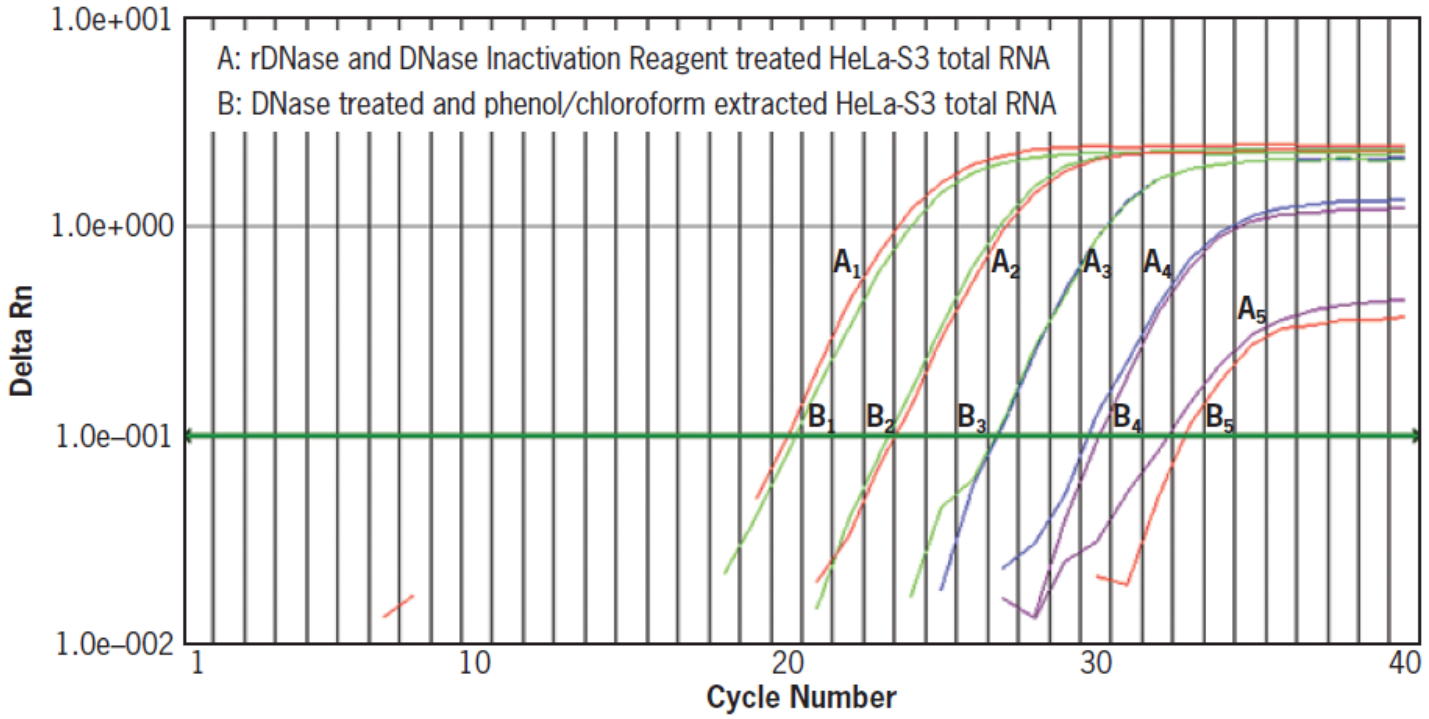


Figure 3 Total RNA treated with DNA-*free*™ reagents is compatible with real-time RT-PCR. 10-fold serial dilutions from 100 ng to 10 pg of HeLa-S3 total RNA was treated with DNA-*free*™ reagents and then the DNase was either removed by following the procedure for routine DNA removal (A1–A5) or was phenol/chloroform 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.)

Troubleshooting

Observation	Possible cause	Recommended action
DNA contamination remains after the initial treatment.	According to a recent publication[1], 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 minutes. 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 6, 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, the DNA-*free*™ Kit treated RNA should be ~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. RT-PCR volumes can be increased to 50 µL or more to accommodate the 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 3, redisperse the DNase Inactivation Reagent by mixing the reaction 2–3 times during the incubation period.

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

Limited product warranty

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Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania

Revision history: Pub. No. 1906M

Revision	Date	Description
F	24 May 2023	Removal of QC text.
E	5 October 2012	Baseline version for DNA-*free*™ Kit.