TURBO DNA-*free*™ Kit

TURBO DNase Treatment and Removal Reagents
Cat #AM1907

Applied Biosystems

A. Product Description

Ambion's TURBO 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 included TURBO DNase (patent pending) is an engineered version of wild type DNase I with 350% greater catalytic efficiency. TURBO DNase has a markedly higher affinity for DNA than conventional DNase I, and is thus more effective in removing trace quantities of DNA contamination. In addition, TURBO DNase maintains up to 50X greater activity than DNase I in solutions containing physiological salt concentrations. The TURBO DNase 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 that contains a small molecule enhancer that extends the activity of the TURBO DNase enzyme by 100-fold or more. Using TURBO DNA-*free*, contaminating DNA is digested to levels below the limit of detection by routine PCR (Figure 1). 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 Table 1). TURBO DNA-*free* treated RNA is suitable for endpoint or real-time RT-PCR, microarray analysis, RPAs, Northerns, and all other RNA analysis methods.

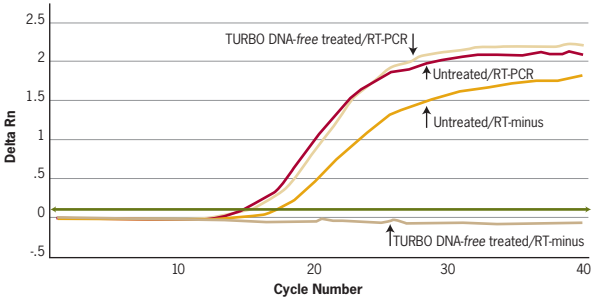


Figure 1. TURBO DNA-*free*™ Reduces Genomic DNA Contamination by Greater than 5 Million Fold.

Equal amounts of mouse spleen total RNA (purified using Ambion's RNAqueous® Kit) were either treated with 7.8 U of TURBO DNase in a 130 µL reaction for 20 min at 37°C, or were left untreated. The digestions were stopped by adding 22 µL DNase Inactivation Reagent. 5 µL (1 µg RNA) was amplified in a one step 25 µL RT-PCR using a TaqMan® primer probe set for mouse GAPDH. Treated and untreated samples were reverse transcribed with Ambion's MessageSensor™ RT Kit. RT-minus samples were subjected to PCR to control for DNA contamination. Results are shown using a linear scale so that the amplification plot for the TURBO DNase-treated, RT-minus sample is visible. The fold-removal (5.4×10^6 fold) of genomic DNA was calculated as follows: The C_t value from the untreated RNA in the RT-minus reaction is the level of gDNA contamination. The fold-removal was determined by subtracting the RT-minus reaction C_t value for the treated RNA sample, 39.5 (the other duplicate's signal was undetectable) from the C_t value of the untreated sample, 17.13, and raising the 17.13 as the exponent with a base of 2.

Table 1. Treatment of RNA with TURBO DNA-*free*™ Maintains Target Sensitivity in Real-time RT-PCR

	Ct for β-actin (duplicates)	
RNA treatment	**100 pg RNA**	**1 pg RNA**
none	24.78 / 24.67	31.83 / 31.53
TURBO DNA-*free* treated	24.50 / 24.62	30.89 /30.88

	Ct for CDC-2 (duplicates)	
none	28.88 / 28.24	34.41 / 35.50
TURBO DNA-*free* treated	27.71 / 28.10	34.04 / 33.99

Total RNA from HeLa S3 cells was treated with the TURBO DNA-*free*™ Kit following the standard protocol. 5 µL of the treated RNA was then reverse transcribed using Ambion's MessageSensor RT Kit, and the resulting cDNA was amplified by real-time RT-PCR using primer and probe sets for either human β-actin or CDC-2 with TaqMan® detection.

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).

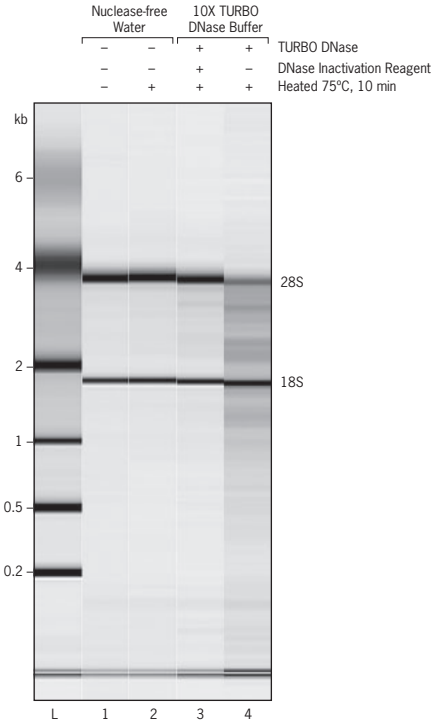


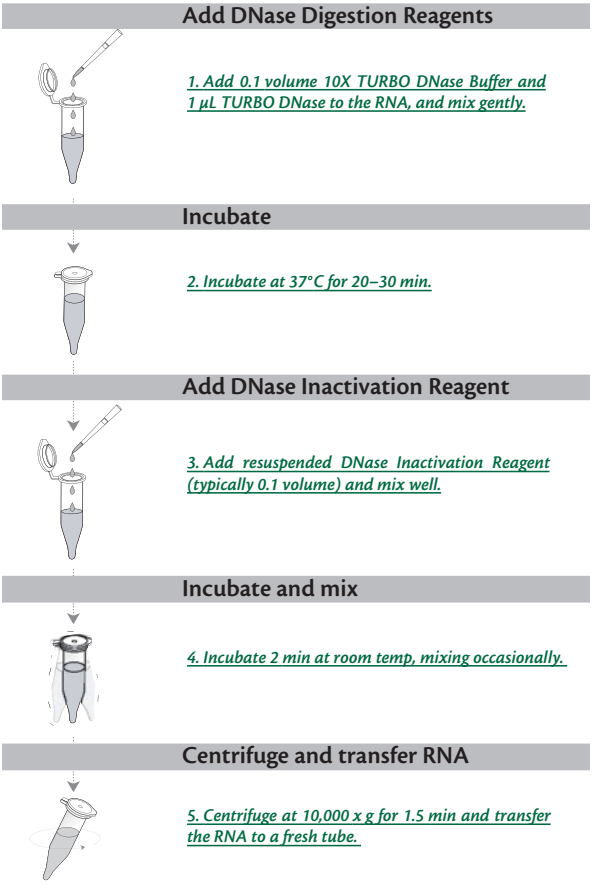
Figure 2. Removal of divalent cations by DNase Inactivation Reagent

HeLa-S3 total RNA (100 ng), in 50 µL 1X TURBO DNase Buffer or in nuclease-free water, was treated with components from the TURBO DNA-*free*™ kit as indicated. Samples were heated for 10 min at 75°C (Lanes 2, 3, & 5), or 3 min at 90°C (Lane 4), to determine if divalent cations from the TURBO DNase Buffer remained in solution, and degraded the RNA. 1 µL of each sample was analyzed on an RNA LabChip® using the Agilent 2100 bioanalyzer. Note that RNA was degraded in the sample that contained TURBO DNase Buffer, but was not treated with the DNase Inactivation Reagent (Lane 5); this degradation is due to the presence of divalent ions that induce heat-mediated RNA cleavage.

B. Procedure Overview

For the detailed protocol, see section E on page 6.

Figure 3. TURBO DNA-*free*™ Procedure Overview



C. How Much RNA Can Be Treated with TURBO DNA-*free*™?

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.

D. TURBO DNA-*free* Components and Storage

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

Amount	Component	Storage
120 µL	TURBO DNase (2 Units/µL)	–20°C
600 µL	10X TURBO DNase Buffer	–20°C
600 µL	DNase Inactivation Reagent	–20°C
1.75 mL	Nuclease-free Water	any temp*

* Store Nuclease-free Water at –20°C, 4°C or room temp

Store the TURBO DNA-*free* Kit at –20°C in a non-frost-free freezer for long-term storage. For convenience, the 10X TURBO DNase Buffer and the DNase Inactivation Reagent can be stored at 4°C for up to 1 week. Properly stored kits are guaranteed for 6 months from the date of shipment.

E. TURBO DNA-*free* Protocol

Protocol Notes

- We recommend conducting reactions in 0.5 mL tubes to facilitate removal of the supernatant after treatment with the DNase Inactivation Reagent.
- TURBO 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.

1. Add 0.1 volume 10X TURBO DNase Buffer and 1 µL TURBO DNase 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 TURBO DNase (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 TURBO DNase Buffer and TURBO DNase. It is also helpful to add only half of the TURBO DNase 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 TURBO DNase 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 TURBO DNA-*free* 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 TURBO DNase was added in step 1, incubate for 30 min, then add the rest of the TURBO DNase 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 TURBO DNase was used in step 1, add 5 µL of DNase Inactivation Reagent.
- For ***rigorous DNase treatments***, where 2–3 µL of TURBO DNase 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 temp, 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 x g for 1.5 min and transfer the RNA to a fresh tube.**

   • For 96 well plates, centrifuge at 2000 x 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.

F. Troubleshooting

1. No RT-PCR product is detectable from RNA treated with TURBO DNA-*free*

DNase Inactivation Reagent could inhibit RT-PCR.

In step .E.5 on page 8, 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.

TURBO DNA-*free* treated RNA should comprise only ~20% of an RT-PCR reaction volume.

For RT-PCR, we recommend that TURBO DNA-*free* treated RNA makes up ~20%, and no more than 40%, of the final RT-PCR volume. Otherwise, components from the TURBO DNase 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 used in RT-PCR should be treated only once with TURBO DNA-*free*.

The salt in TURBO DNA-*free* reactions is carefully balanced for optimal TURBO DNase activity. Subjecting RNA to a second TURBO DNA-*free* treatment will introduce additional salts that could interfere with downstream enzymatic reactions. If a second DNase treatment is required, please refer to the "TURBO DNA-*free* 2nd Digest Protocol" available online at:

www.ambion.com/techlib/append/supp/digest.html

2. 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 during step E.4 on page 7, redisperse the DNase Inactivation Reagent by mixing the reaction 2–3 times during the incubation period.

3. The RNA absorbance spectrum has an unusual profile after treatment with TURBO DNA-*free*.

If the concentration of RNA in the sample is less than about 50 ng/µL, you may notice significant absorbance at ~230 nm. A_{260}/A_{280} ratios may also be slightly lower than normal when the RNA concentration is ≤25 ng/µL. These differences in the absorbance profile are caused by the enhancer in the TURBO DNase Buffer. Exhaustive comparisons at Ambion with both treated and untreated RNA samples indicate that the enhancer has no affect on accurate RNA quantification unless the RNA concentration is below 10 ng/µL. For more information, please see:

www.ambion.com/catalog/supp/absorbance.html

G. TURBO DNA-*free* Specifications

Contents:

Amount	Component	Storage
120 µL	TURBO DNase (2 Units/µL)	–20°C
600 µL	10X TURBO DNase Buffer	–20°C
600 µL	DNase Inactivation Reagent	–20°C
1.75 mL	Nuclease-free Water	any temp*

* Store Nuclease-free Water at –20°C, 4°C, or room temp

Storage conditions:

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

To obtain Material Safety Data Sheets

- Material Safety Data Sheets (MSDSs) can be printed or downloaded from product-specific links on our website at the following address:

  www.ambion.com/techlib/msds

- Alternatively, e-mail your request to MSDS_Inquiry_CCRM@appliedbiosystems.com. Specify the catalog or part number(s) of the product(s), and we will e-mail the associated MSDSs unless you specify a preference for fax delivery.
- For customers without access to the internet or fax, our technical service department can fulfill MSDS requests placed by telephone or postal mail. (Requests for postal delivery require 1–2 weeks for processing.)

H. Quality Control

Functional testing

The activity of the TURBO DNase 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 TURBO DNase and TURBO DNase Buffer components. Results are analyzed by agarose gel electrophoresis and the Agilent 2100 bioanalyzer, respectively.

Nuclease testing

Kit components are tested in the following nuclease assays:

RNase activity

Meets or exceeds specification when a sample is incubated with ^{32}P-labeled RNA and analyzed by PAGE.

Nonspecific endonuclease activity

Meets or exceeds specification when a sample is incubated for 14–16 hr with 300 ng supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.

Exonuclease activity

Meets or exceeds specification when a sample is incubated for 14–16 hr with 40 ng ^{32}P-labeled *Sau*3A fragments of pUC19 and analyzed by PAGE.

Protease testing

Meets or exceeds specification when a sample is incubated for 14–16 hr with 1 µg protease substrate and analyzed by fluorescence.

Manual 1907M Revision C **Revision Date: October 25, 2007**

For research use only. Not for use in diagnostic procedures. By use of this product, you accept the terms and conditions of all applicable Limited Label Licenses. For statement(s) and/or disclaimer(s) applicable to this product, see below.

Literature Citation: When you are describing a procedure utilizing this product in a Materials and Methods Section for publication, we would appreciate that you refer to it as the TURBO DNA-*free*™ Kit.

Warranty and Liability: Applied Biosystems is committed to delivering superior product quality and performance, supported by industry-leading global service and technical support teams. Warranty information for the accompanying consumable product is available at www.ambion.com/info/warranty in "Limited Warranty for Consumables," which is subject to the exclusions, conditions, exceptions, and limitations set forth under the caption "EXCLUSIONS, CONDITIONS, EXCEPTIONS, AND LIMITATIONS" in the full warranty statement. Please contact Applied Biosystems if you have any questions about our warranties or would like information about post-warranty support.

Patents and Licensing Notifications: This product is compatible with the 5' nuclease detection and dsDNA-binding dye processes covered by patents owned or licensable by Applied Biosystems. No license under these patents is conveyed expressly, by implication, or by estoppel to the purchaser by the purchase of this product. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

Ambion/AB Trademarks: Applied Biosystems, AB (Design), ABI Prism and Applera are registered trademarks of Applera Corporation or its subsidiaries in the US and/or certain other countries. Ambion, RNAqueous and The RNA Company are registered trademarks; and DNA-*free* and MessageSensor are trademarks of Ambion, Inc., in the U.S. and/or certain other countries. TaqMan is a registered trademark of Roche Molecular Systems, Inc
All other trademarks are the sole property of their respective owners.

Applied Biosystems
Ambion®

U.S./Canada
(English) 800-327-3002
(French) 800-668-6913
Fax +1-512-651-0201

Europe
tel +44 (0)1480-373-020
fax +44 (0)1480-373-010

Japan
tel 81 (0) 3 5566 6230
fax 81 (0) 3 5566 6507

Direct free phone numbers, distributors:
www.appliedbiosystems.com