TURBO DNA-*free*™ Kit

TURBO 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

TURBO DNA-*free*™ DNase Treatment and Removal Reagents are designed to remove contaminating DNA from RNA preparations, and subsequently remove DNase and divalent cations from the sample. The TURBO DNase™ enzyme is an engineered version of wild type DNase I with 350% greater catalytic efficiency and a markedly higher affinity for DNA than conventional DNase I, making it more effective in removing trace quantities of DNA contamination . TURBO DNase™ enzyme is also capable of maintaining up to 50X greater activity than DNase I in solutions at physiological salt concentrations.

The enzyme is expressed in an animal-free system, and extensively purified in a bovine-free process. It is guaranteed to lack contaminating RNase activity. The kit includes DNase reaction buffer containing a small molecule enhancer to extend 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 (See Figure 1 on page 4). The DNase is subsequently removed using a novel method which does not require phenol/chloroform extraction, alcohol precipitation, heating, or the addition of EDTA (See Table 1). 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 (See Figure 2 on page 5).

TURBO DNA-*free*™ treated RNA is suitable for endpoint or real-time RT-PCR, microarray analysis, RPAs, northern blot, and all other RNA analysis methods.

How much RNA can be treated with TURBO DNA-*free*™ reagents?

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.

Contents and storage

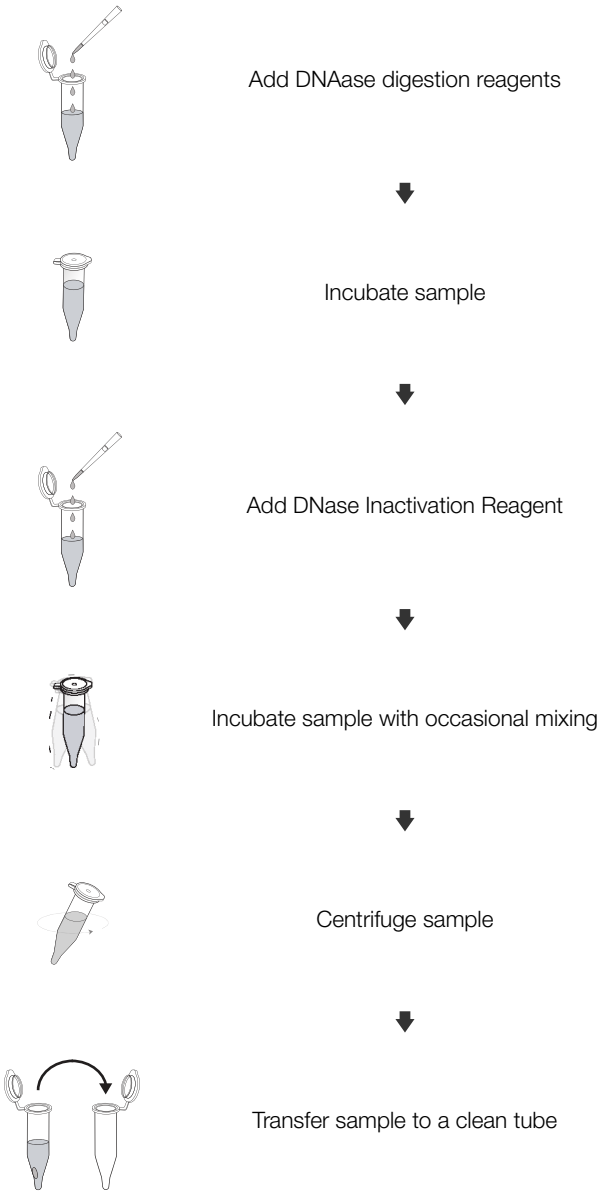
Reagents are provided for 50 TURBO DNA-*free*™ treatments (up to 100 µL each).

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

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.

Procedure overview

For the detailed procedure, see “Perform routine DNase treatment” on page 3.



TURBO DNA-*free*™ procedure

Procedural guidelines

- A typical reaction volume is 50 µL, but reactions volumes can range from 10–100 µL.
- 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 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 (see “Perform routine DNase treatment” on page 3).
  - **Rigorous DNase treatment:** Sample contains >200 µg nucleic acid per mL or RNA that is severely contaminated with DNA (i.e., >2 µg DNA/50 µL). These sample can be diluted prior to treatment, or if the sample cannot be diluted, simply increase the amount of TURBO DNase™ Enzyme to 2–3 µL (4–6 U). See “Perform rigorous DNase treatment” on page 3. 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 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.

Perform routine DNase treatment

1. Add 0.1 volume 10X TURBO DNase™ Buffer and 1 µL of TURBO DNase™ Enzyme 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 TURBO DNase™ 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 the sample for 5 minutes at room temperature. Flick the tube 2–3 times during the incubation period to keep the DNase Inactivation Reagent suspended.

   Note: If room temperature cools below 22–26°C, move the tubes to a heat block or oven to control the temperature. Cold environments can inhibit inactivation of the TURBO DNase™ Enzyme, leaving residual DNase in the RNA sample.
6. Centrifuge the samples, then carefully transfer the supernatant containing the RNA to a fresh tube. Do not disturb the DNase Inactivation Reagent pellet.
   - Centrifuge microcentrifuge tubes at 10,000 × *g* for 1.5 minutes.
   - Centrifuge 96-well plates at 2000 × g for 5 minutes.

   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 TURBO DNase™ Buffer to the RNA, then mix gently.
3. Add TURBO DNase™ Enzyme to the RNA, then mix gently.
   - Use 1 µL of TURBO DNase™ Enzyme for diluted samples.
   - Use 2–3 µL (4–6 U) of TURBO DNase™ Enzyme 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 TURBO DNase™ Enzyme to the sample.
4. Incubate samples 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 the TURBO DNase™ Enzyme from the previous step, 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 0.2 volumes of resuspended DNase Inactivation Reagent, then mix well.
7. Incubate the sample for 5 minutes at room temperature. Flick the tube 2–3 times during the incubation period to keep the DNase Inactivation Reagent suspended.

   Note: If room temperature cools below 22–26°C, move the tubes to a heat block or oven to control the temperature. Cold environments can inhibit inactivation of the TURBO DNase™ Enzyme, leaving residual DNase in the RNA sample.
8. Centrifuge the samples, then carefully transfer the supernatant containing the RNA to a fresh tube. Do not disturb the DNase Inactivation Reagent pellet.
   - Centrifuge microcentrifuge tubes at 10,000 × *g* for 1.5 minutes.
   - Centrifuge 96-well plates at 2000 × g for 5 minutes.

   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

Reduction of gDNA contamination

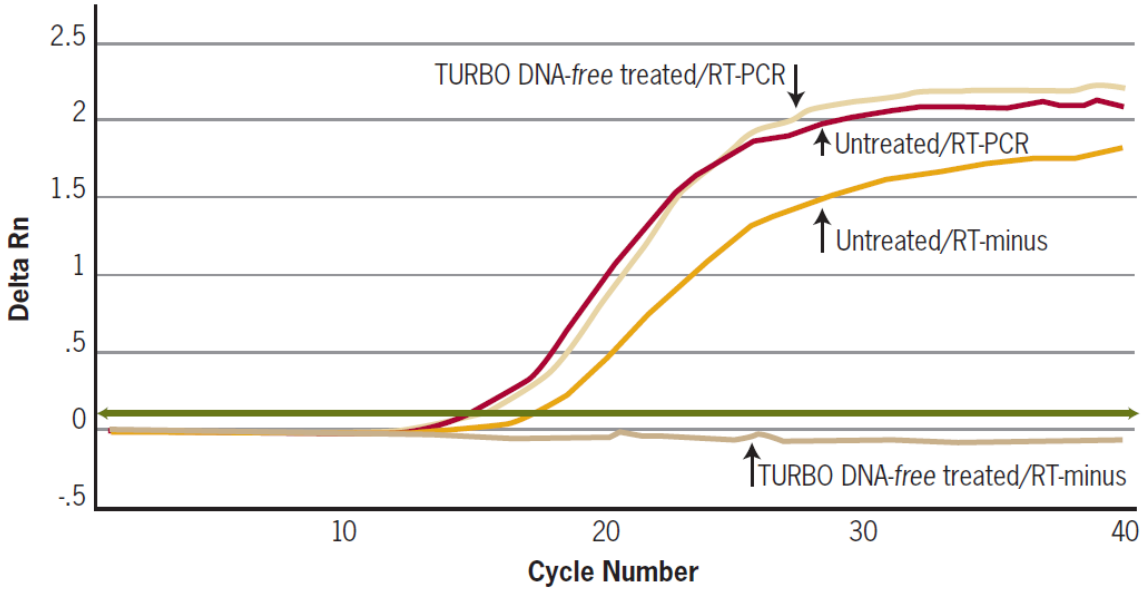


Figure 1 TURBO DNA-*free*™ reduces genomic DNA contamination by >5 million fold.

Equal amounts of mouse spleen total RNA (purified using the RNAqueous™ kit) were either treated with 7.8 U of TURBO DNA-*free*™ in a 130 µL reaction for 20 min at 37°C, or left untreated. Digestion was stopped by adding 22 µL DNase inactivation reagent. 5 µL (1 µg RNA) of sample was amplified in a one step 25-µL RT-PCR reaction using a TaqMan™ primer probe set for mouse GAPDH.

Treated and untreated samples were reverse transcribed with the 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.

Target sensitivity after treatment

Table 1 Treatment of RNA with TURBO DNA-*free*™ maintains target sensitivity in real-time RT-PCR.

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 the 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.

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

Removal of divalent cations

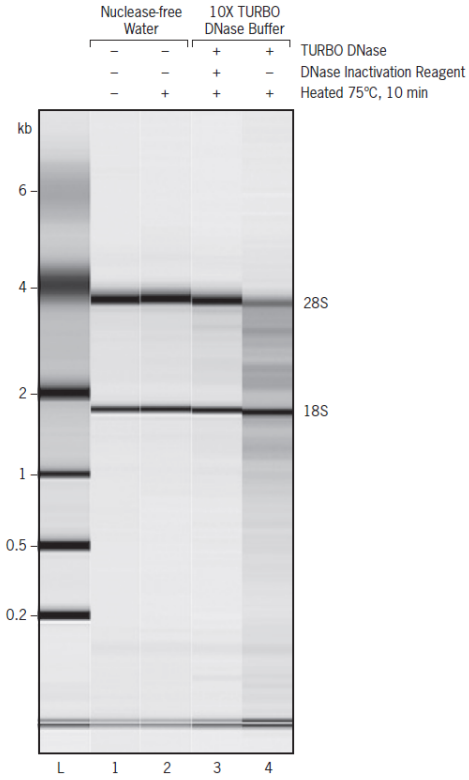


Figure 2 Removal of divalent cations by DNase inactivation reagents.
HeLa-S3 total RNA (100 ng), in 50 µL 15 TURBO DNase™ buffer or in nuclease-free water, was treated with components from the TURBO DNA-*free*™ kit as indicated. Samples were heated for 10 min at 75°C (lanes 2, 3, & 5), or 3 min at 90°C (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™ Instrument. 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.

Troubleshooting

Observation	Possible cause	Recommended action
No RT-PCR product is detectable from treated RNA	DNase Inactivation Reagent is inhibiting RT-PCR.	Do not disturb the DNase Inactivation Reagent pellet when transferring the RNA to a new tube after centrifugation. It may be necessary 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.
	Components from the TURBO DNase™ Buffer and the DNase Inactivation Reagent are interfering with the reaction.	The recommended volume of TURBO DNA-*free*™ treated RNA is ~20% of an RT-PCR reaction volume, and no more than 40%, of the final RT-PCR volume. If necessary, RT-PCR volumes can be increased to 50 µL or more to accommodate your RNA without exceeding the 20–40% limit.
	TURBO DNA-*free*™ treatment used on RNA more than once. The salt in TURBO DNA-*free*™ reactions is carefully balanced for optimal TURBO DNase™ activity. Subjecting RNA to a second TURBO DNA-*free*™ treatment introduces additional salts that can interfere with downstream enzymatic reactions.	Do not perform TURBO DNA-*free*™ treatment on RNA to be used in RT-PCR (or other salt sensitive reactions) more than once
RNA is degraded upon heating to > 60°C	Divalent cations were not completely removed from samples. 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, redisperse the DNase Inactivation Reagent by mixing the reaction 2–3 times over the course of incubation.
The RNA absorbance spectrum has an unusual profile after TURBO DNA-*free*™ treatment.	Differences in the absorbance profile are caused by the enhancer in the TURBO DNase™ Buffer. If the concentration of RNA in the sample is less than about 50 ng/µL, there can be 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.	Exhaustive comparisons 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.

Limited product warranty

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

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Revision	Date	Description
J	7 June 2023	Removal of QC text.
H	29 June 2018	Baseline version for TURBO DNA-*free*™ Kit.