# DNase Treatment of RNA

# 18

Isabella Dotti and Serena Bonin

## Contents



## 18.1 Introduction and Purpose

A frequent cause of false positives in RT-PCR-based assays is the amplification of genomic DNA (gDNA) contaminating RNA preparations. Because of PCR sensitiveness, a single copy of a genomic sequence can be theoretically detected. This phenomenon is exacerbated when tested genes present pseudogenes at the DNA level, which have sequences similar to mRNA (information about pseudogenes can be obtained in the web[1]). For this reason DNA removal by DNase digestion is often a necessary step. Moreover, what has been observed is a decrement in real-time threshold cycles in cDNA obtained from RNA extracts that was treated with DNase prior to reverse transcription, in comparison with the undigested ones [1]. The good outcome of the DNase digestion can be then checked by performing an RT-PCR assay in which the reverse transcriptase is not included.

The following protocols describe two different DNase treatments that can be chosen on the basis of RNA availability.

## 18.2 DNase Digestion Followed by Phenol/Chloroform Extraction

This protocol allows complete removal of DNase enzyme after DNA digestion [2]. The adjustable volumes of reaction allow digestion of even large amounts

[1]Pseudogenes are DNA non-functional sequences present in the genome that have strong similarities to mRNA but, in general, are unable to be transcribed. The nonfunctionality of the pseudogenes is often caused by the lack of functional promoters or other regulatory elements. Pseudogenes are quite difficult to identify, because their characterization is performed through sequence calculations and alignments rather than biologically proven. A comprehensive list of identified pseudogenes can be found at http://www.pseudogene.org.

I. Dotti (✉) and S. Bonin
Department of Medical, Surgical and Health Sciences,
University of Trieste, Cattinara Hospital,
Strada di Fiume 447, Trieste, Italy

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of DNA, also when RNA concentration is very low. This protocol is recommended when an RNA solution is not pure (presence of co-extracted contaminants) or when a high RNA quantity should be used in the following analyses. It is not the best choice when the starting amount of RNA is very low (for example, when recovered from small biopsies or after microdissection). In such case, Protocol 18.3 is suggested.

### 18.2.1 Reagents

Reagents from specific companies are reported here, but reagents of equal quality purchased from other companies may be used.

All reagents should be RNase free[2] and DEPC treated

- *DEPC-treated water (DEPC $H_2O$)*: add 1 ml of diethylpirocarbonate (DEPC) (0.1% final concentration) to 1 l of sterile water at 37°C overnight under fume hood and then autoclave[3]
- *10 U/µl DNaseI FPLC pure* (cod. 27-0514 GE Healthcare)
- *10× Reaction buffer*: 400 mM Tris-HCl (pH 7.5), 60 mM $MgCl_2$
- *Phenol/$H_2O$*
- *Chloroform*
- *0.8 M LiCl in absolute EtOH*
- *1 mg/ml Glycogen*
- *Absolute EtOH*

### 18.2.2 Equipment

- *Adjustable pipettes*,[4] range: 2–20 µl, 20–200 µl, 100–1,000 µl
- *Nuclease-free aerosol-resistant pipette tips*
- *1.5 ml tubes and 0.2 ml tubes* (autoclaved)
- *Thermomixer* (e.g., Eppendorf)

### 18.2.3 Method

- A 20 µl reaction volume is prepared, adding the following components in a 1.5 ml tube:

| | |
|---|---|
| 10 × Reaction buffer | 2 µl |
| 10 U/µl DNaseI | 1 µl |
| Total RNA | 2 µg[5] |
| DEPC $H_2O$ | to 20 µl final[6] |

- Incubate the mixture at 37°C for 20′. Spin down the condensate from the tube walls.
- Bring the final volume to 100 µl with DEPC-treated $H_2O$ and add 70 µl phenol/$H_2O$ + 30 µl chloroform.[7] Vortex, keep on ice for 20′ and spin the tube for 20′ at 14,000 rpm in a microcentrifuge at 4°C.
- Carefully transfer the supernatant to a new tube (avoid touching the interphase), and add three volumes of 0.8 M LiCl in absolute EtOH and 5 µl of glycogen solution. Mix the solution by inversion.
- Leave the solution at –20°C for 24 h.
- Spin the tube for 20′ at 14,000 rpm in a microcentrifuge at 4°C; remove the supernatant without disturbing the pellet.
- Wash the pellet with 100 µl ice cold absolute EtOH and spin the tube at 14,000 rpm for 10′ at 4°C.
- Remove the supernatant, air dry the RNA pellet and resuspend it in the proper amount of DEPC-treated $H_2O$.
- Determine the new RNA concentration photometrically at 260 and 280 nm, adding 1 µl RNA solution to 199 µl of $H_2O$.[8]

[2]Sterile, disposable plasticware should preferably be used because it is RNase free. If general laboratory glassware or plasticware is used, it should be pre-soaked in 0.1% DEPC-treated $H_2O$ for 2 h at 37°C. The DEPC-treated items should be rinsed thoroughly with DEPC $H_2O$ and then autoclaved. Alternatively, glassware could be put in an oven at 250°C for 2 h.

[3]After this treatment the water is essentially free from RNases and can be used to prepare solutions or to rinse any items to use for RNA isolation. DEPC is a carcinogen and should be handled with care under a fume hood.

[4]Clean the pipettes with a disinfectant (e.g., Meliseptol®rapid) and leave them under the UV lamp for at least 10 min. Alternatively it is possible to autoclave the pipette depending on the provider instructions. Pipettes dedicated only to RNA extraction and treatment should be preferably used.

[5]If more than 5 µg RNA is digested, increase the DNase units. If a larger amount of RNA is treated, scale up the reaction volume because the viscosity of the solution may increase, preventing DNase activity.

[6]The volume of reaction can be increased up to 100 µl when, for example, the starting RNA is resuspended in large volumes; in such case, the DNase unit concentration has to be maintained by increasing the quantity.

[7]Alternatively, it is possible to purify DNase-treated RNA by the use of silica columns or the direct performance of an on-column DNase digestion (for details, see RNase free DNase set on http://www.qiagen.com/).

[8]See Chap. 16 for details.

- The solution is ready to be used for RT-PCR. Alternatively, RNA can be stored at –80°C until use; it is better if it is divided into aliquots to avoid repeated freezing and thawing.

## 18.3 DNase Digestion Followed by Heat Inactivation

This protocol can be used when the starting amount of RNA is low and DNase removal by extraction could determine its complete loss [3].

### 18.3.1 Reagents

Reagents from specific companies are here reported, but reagents of equal quality purchased from other companies may be used.

All reagents should be RNase free[9] and DEPC treated

- *DEPC-treated water (DEPC $H_2O$)*: Add 1 ml of diethylpirocarbonate (DEPC) (0.1% final concentration) to 1 l of sterile water at 37°C overnight and then autoclave[10]
- *10 U/µl DNaseI FPLC pure* (cod. 27-0514 GE Healthcare)
- *10× Reaction buffer*: 400 mM Tris-HCl (pH 7.5), 60 mM $MgCl_2$
- *25 mM EDTA*[11]

### 18.3.2 Equipment

- *Adjustable pipettes*,[12] range: 2–20 µl, 20–200 µl, 100–1,000 µl
- *Nuclease-free aerosol-resistant pipette tips*
- *1.5 ml tubes and 0.2 ml tubes* (autoclaved)
- *Thermomixer* (Eppendorf)

### 18.3.3 Method

- Hereafter, a 20 µl reaction volume is reported, adding the following components in a 1.5 ml tube:

| | |
|---|---|
| 10 × Reaction buffer | 2 µl |
| 10 U/µl DNaseI | 1 µl |
| Total RNA | 2 µg[13] |
| DEPC $H_2O$ | to 20 µl final[14] |

- Incubate the mixture at 25°C for 15′.[15] Spin the tube to collect the condensate from the tube walls.
- Add 2 µl of 25 mM EDTA to the solution; mix and heat at 65°C for 10′ to inactivate DNase. Immediately, chill the solution on ice and spin the tube.
- Determine the RNA concentration photometrically at 260 and 280 nm, adding 1 µl RNA solution to 199 µl of $H_2O$.[16] Alternatively, for a more approximate quantification, determine the new RNA concentration simply taking into account the starting RNA concentration and the reaction volume before and after DNase treatment.

[9]Sterile, disposable plasticware should be preferably used because it is RNase free. If general laboratory glassware or plasticware is used, it should be pre-soaked in 0.1% DEPC-treated $H_2O$ for 2 h at 37°C. The DEPC-treated items should be rinsed thoroughly with DEPC $H_2O$ and then autoclaved. Alternatively, glassware could be put in an oven at 250°C for at least 2 h.

[10]After this treatment, the water is essentially free from RNases and can be used to prepare solutions or to rinse any items to be used for RNA isolation. DEPC is a carcinogen and should be handled with care under a fume hood.

[11]The EDTA helps to protect RNA at high temperatures.

[12]Clean the pipettes with a disinfectant (e.g., Meliseptol®rapid) and leave them under the UV lamp for at least 10 min. Alternatively it is possible to autoclave the pipette according to the provider instructions. Pipettes dedicated only for RNA extraction and treatment should be preferably used.

[13]If more than 4 µg RNA are digested, increase DNase units. If a larger amount of RNA is digested, scale up the reaction volume as more RNA may increase the viscosity of the solution preventing DNase activity.

[14]The volume of reaction can be increased up to 100 µl when the starting RNA is resuspended in large volumes; in this case also the DNase units must be increased.

[15]This incubation time and temperature has been shown to be sufficient to remove contaminant DNA (manufacturer's protocol suggests 10–20' at 37°C).

[16]For spectrophotometric quantification of RNA after DNase treatment remember to use a DNase solution complete of buffer and EDTA without RNA as a blank.

- The mixture can be used directly for RT-PCR. Alternatively, RNA can be stored at –80°C until use; it is better if it is divided into aliquots.

## References

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