

# RQ1 RNase-Free DNase



Technical Bulletin No. 518

INSTRUCTIONS FOR USE OF PRODUCT M6101. PLEASE DISCARD PREVIOUS VERSIONS.

All technical literature is available on the Internet at [www.promega.com](http://www.promega.com)

Please visit the web site to verify that you are using the most current version of this Technical Bulletin.

I. Description .....	1
II. Product Components .....	2
III. DNase Treatment of RNA Samples Prior to RT-PCR .....	2
IV. Other Applications.....	3
A. Transcription .....	3
B. Nick Translation.....	3
C. Transcription Factor DNase I Footprinting .....	3
D. Production of Random Fragments.....	3
V. Miscellaneous Information .....	3
VI. Composition of Buffers and Solutions .....	4
VII. References .....	4

## I. Description

RQ1 (RNA-Qualified) RNase-Free DNase is a DNase I that degrades both double-stranded and single-stranded DNA endonucleolytically, producing 3'-OH oligonucleotides (1). (RQ1 RNase-Free DNase may be used in applications where maintaining the integrity of the RNA is critical.) This DNase is used for applications such as nick translation (2), production of random fragments (3), cleavage of genomic DNA for footprinting (3), removal of DNA template after in vitro transcription (4), and removal of DNA from RNA samples prior to applications such as RT-PCR (5).

In the presence of  $Mg^{2+}$ , DNase I attacks each strand of DNA independently, and the sites of cleavage are distributed in a statistically random fashion (Figure 1; 6). In the presence of  $Mn^{2+}$ , DNase I cleaves both strands of DNA at approximately the same site to yield fragments that are blunt ended or have protruding termini only one or two nucleotides in length (6).

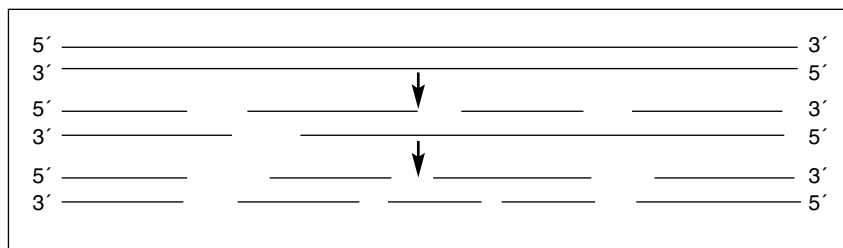


Figure 1. Endonuclease activity of DNase I in the presence of  $Mg^{2+}$ .





**CAUTION:**

Do not store in a frost-free freezer. RQ1 RNase-Free DNase **does not** contain an RNase inhibitor. Handle carefully to avoid RNase contamination.



**SALTS**

in the RQ1 DNase Reaction Buffer and Stop Solution cause aberrant migration of the RNA on gels. Phenol:chloroform extraction should be performed after step 2 if gel electrophoresis of the RNA is desired.

## II. Product Components

Product	Concentration	Size	Cat.#
RQ1 RNase-Free DNase	1 unit/ $\mu$ l	1,000u	M6101

Includes:

- 1000u RQ1 RNase-Free DNase
- 1ml RQ1 DNase 10X Reaction Buffer
- 1ml RQ1 DNase Stop Solution
- 1 Protocol

**Storage Conditions:** Store at  $-20^{\circ}\text{C}$ . RQ1 RNase-Free DNase is supplied in storage buffer containing 10mM HEPES (pH 7.5), 50% (v/v) glycerol, 10mM  $\text{CaCl}_2$  and 10mM  $\text{MgCl}_2$ . Do not store in a frost-free freezer. Avoid multiple freeze-thaw cycles.

## III. DNase Treatment of RNA Samples Prior to RT-PCR

1. Set up the DNase digestion reaction as follows:

RNA in water or TE buffer	1–8 $\mu$ l
RQ1 RNase-Free DNase 10X Reaction Buffer	1 $\mu$ l
RQ1 RNase-Free DNase (see Note 1)	<u>1u/<math>\mu</math>g RNA</u>
Nuclease-free water to a final volume of	10 $\mu$ l

2. Incubate at  $37^{\circ}\text{C}$  for 30 minutes.

**Note:** If performing gel electrophoresis on RNA samples, it is necessary to perform a phenol:chloroform extraction and ethanol precipitation before loading the samples on the gel. Steps 3 and 4 may be omitted if a phenol:chloroform extraction is performed.

3. Add 1 $\mu$ l of RQ1 DNase Stop Solution to terminate the reaction.
4. Incubate at  $65^{\circ}\text{C}$  for 10 minutes to inactivate the DNase.
5. Add all, or a portion of, the treated RNA to the RT-PCR reaction. See the *Access RT-PCR System*<sup>(a)</sup> *Technical Bulletin* #TB220.

**Notes:**

1. Use 1 unit of RQ1 RNase-Free DNase per microgram of RNA. For smaller amounts of RNA, use 1 unit of RQ1 RNase-Free DNase per reaction.
2. The RQ1 RNase-Free DNase digestion contains a final concentration of 10mM  $\text{MgSO}_4$ . **When adding the DNase-treated RNA to an RT-PCR reaction, carryover of magnesium must be considered.** For example, the addition of 1 $\mu$ l of treated RNA to a 50 $\mu$ l RT-PCR reaction will raise the magnesium concentration by 0.2mM, and the addition of 5 $\mu$ l of treated RNA will raise the magnesium concentration by 1mM. The requirement for magnesium may be different in the RQ1 DNase digestion step and in the amplification reaction.
  - RQ1 DNase activity increases as the  $\text{Mg}^{2+}$  concentration increases up to 5–10mM. At a concentration of 1mM  $\text{Mg}^{2+}$ , RQ1 DNase is expected to be at least four-fold less active than at the optimal  $\text{Mg}^{2+}$  concentration.
  - For some templates, the yield from the amplification reaction is highly dependent on the  $\text{Mg}^{2+}$  concentration, and the optimal  $\text{Mg}^{2+}$  concentration may be as low as 1mM.

If an increased  $Mg^{2+}$  concentration is not tolerable in the amplification reaction, the following alternatives may be used.

- The RQ1 RNase-Free DNase 10X Reaction Buffer may be diluted 1:10 with 400mM Tris (pH 8.0), 10mM  $CaCl_2$  prior to DNase digestion. (Note that, under these conditions, the RQ1 DNase will be approximately four-fold less active than under standard reaction conditions.)
- An alternative DNase reaction buffer may be used (such as the RT or PCR reaction Buffer) if that buffer contains at least 1mM  $Mg^{2+}$ .
- The RNA sample may be diluted in water prior to RT-PCR to codilute the  $MgSO_4$  to a concentration that is compatible with this application.
- The RNA may be purified with a standard Phenol:Chloroform extraction followed by an ethanol precipitation.

#### IV. Other Applications

RQ1 RNase-Free DNase may be used in a number of other applications where maintaining the integrity of RNA is important. These include in vitro transcription, nick translation and transcription factor DNase I footprinting.

##### A. Transcription

To remove template DNA, RQ1 RNase-Free DNase may be added directly to the transcription reaction. Please refer to the *Riboprobe® in vitro Transcription Systems<sup>(b)</sup> Technical Manual #TM016 (4)* for specific protocol information.

##### B. Nick Translation

For protocol information on the use of RQ1 RNase-Free DNase for this application, please refer to the *Nick Translation System Technical Bulletin #TB044 (2)*.

##### C. Transcription Factor DNase I Footprinting

RQ1 RNase-Free DNase is a component of Promega's Core Footprinting System and may be used in footprinting experiments to determine whether a gene of interest contains a specific DNA binding protein binding site. For specific protocol information, please refer to the *Core Footprinting System Technical Bulletin #TB137* or see reference 7.

##### D. Production of Random Fragments

For protocol information on the use of DNase I for random fragmentation of DNA, see reference 6.

#### V. Miscellaneous Information

**Source:** Bovine pancreas.

**Molecular Weight:** 31,000 Daltons.

**Inhibitors:** EGTA; EDTA (8); salt concentrations >100mM will result in reduction of DNase activity.

**Heat Inactivation:** 10 minutes at 65°C in the presence of Stop Solution.

**Requirement:**  $Ca^{2+}$  and  $Mg^{2+}$  or  $Mn^{2+}$  (8).

**Unit Definition:** One unit of RQ1 RNase-Free DNase is the amount of enzyme required to completely degrade 1µg of lambda DNA in 10 minutes at 37°C in 50µl of

buffer containing 40mM Tris-HCl (pH 7.9), 10mM NaCl, 6mM MgCl<sub>2</sub> and 10mM CaCl<sub>2</sub>. Under these assay conditions 1 unit of RQ1 RNase-Free DNase activity is approximately equal to 1 Kunitz unit.

**Note:** Under different buffer conditions, the amount of RQ1 RNase-Free DNase required to completely digest a given amount of DNA must be empirically determined. For example, salt concentrations >100mM will result in reduction of DNase activity. Ca<sup>2+</sup> and Mg<sup>2+</sup> are essential for RQ1 DNase activity.

## VI. Composition of Buffers and Solutions

### RQ1 DNase 10X Reaction Buffer

400mM Tris-HCl (pH 8.0)  
100mM MgSO<sub>4</sub>  
10mM CaCl<sub>2</sub>

### RQ1 DNase Stop Solution

20mM EGTA (pH 8.0)

## VII. References

1. Moore, S. (1981) *Pancreatic DNase In: The Enzymes*, Volume 14A, P.D. Boyer, Ed., Academic Press, New York, 281.
2. *Nick Translation System Technical Bulletin #TB044*, Promega Corporation.
3. Cobianchi, F. and Wilson S.H. (1987) Enzymes for modifying and labeling DNA and RNA. *Meth. Enzymol.* **152**, 94.
4. *Riboprobe® in vitro Transcription Systems Technical Manual #TM016*, Promega Corporation.
5. *Access RT-PCR System and Access RT-PCR Introductory System Technical Bulletin #TB220*, Promega Corporation.
6. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual 2nd ed.*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
7. Ausubel, F.M. (1994) *Current Protocols in Molecular Biology*, John Wiley and Sons, New York, 12.4.
8. Ausubel, F.M. (1994) *Current Protocols in Molecular Biology*, John Wiley and Sons, New York, 3.12.

(a)The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

(b)U.S. Pat. No. 5,552,302, European Pat. No. 0 422 217 and Australian Pat. No. 646803 have been issued to Promega Corporation for the methods and compositions for production of human recombinant placental ribonuclease inhibitor.

© 1991–2000 Promega Corporation. All Rights Reserved.

RiboClone is a trademark of Promega Corporation and is registered with the U.S. Patent and Trademark Office.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.



### Promega Corporation

2800 Woods Hollow Road	
Madison, WI 53711-5399	USA
Telephone	608-274-4330
Fax	608-277-2516
Internet	<a href="http://www.promega.com">www.promega.com</a>