Product Contents

RQ1 RNase-Free DNase:

Part No.	Size (units)
M610A	1,000

Description: RQ1 (RNA-Qualified) RNase-Free DNase is a DNase I (endonuclease) that degrades both double-stranded and single-stranded DNA, 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 suited 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 (6). In the presence of Mn^{2+}, DNase I cleaves both strands of DNA at approximately the same site to yield fragments with blunt ends or protruding termini of one or two nucleotides in length (6).

10X Reaction Buffer (M198A): The RQ1 DNase 10X Reaction Buffer provided with this enzyme has a composition of 400mM Tris-HCl (pH 8.0), 100mM $MgSO_4$ and 10mM $CaCl_2$.

Enzyme Storage Buffer: RQ1 DNase is supplied in 10mM HEPES (pH 7.5), 50% glycerol (v/v), 10mM $CaCl_2$ and 10mM $MgCl_2$.

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

Inhibitors: EGTA; EDTA (7); salt concentrations >100mM will reduce DNase activity.

Molecular Weight: 31,000 Daltons.

Requirement: Ca^{2+} and Mg^{2+} or Mn^{2+} (7).

Source: Bovine pancreas.

Storage Temperature: Store at –20°C. Avoid exposure to frequent temperature changes. See the expiration date on the Product Information Label.

Stop Solution (M199A): 20mM EGTA (pH 8.0).

Unit Definition: One unit of RQ1 RNase-Free DNase is defined as the amount required to completely degrade 1µg of lambda DNA in 10 minutes at 37°C in 50µl of a buffer containing 40mM Tris-HCl (pH 7.9), 10mM NaCl, 6mM $MgCl_2$ and 10mM $CaCl_2$. Under these assay conditions one unit of RQ1 DNase activity is approximately equal to one Kunitz unit. See the unit concentration on the Product Information Label.

Usage Notes:

1. This DNase solution does not contain an RNase inhibitor. Observe caution in handling the product to ensure against contaminating it with RNase.
2. Under different buffer conditions the amount of DNase required to completely digest a given amount of DNA may need to be empirically determined. For example, salt concentrations >100mM will reduce DNase activity.

Quality Control Assays

Contaminant Activity

RNase Assay: To test for RNase activity, 50ng of [^{3}H]RNA is incubated with 5 units of RQ1 RNase-Free DNase in Transcription Optimized 1X Buffer (Cat.# P1181, diluted fivefold) for 1 hour at 37°C, and the release of radiolabeled nucleotides is monitored by scintillation counting of TCA-soluble material. The minimum passing specification is <3% release.

[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, Australian Pat. No. 646803 and Japanese Pat. Nos. 3009458 and 3366596 have been issued to Promega Corporation for the methods and compositions for production of human recombinant placental ribonuclease inhibitor.

[c]U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687, Australian Pat. Nos. 616881 and 641261 and other pending and issued patents, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.

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Usage Information

I. DNase Treatment of RNA Samples Prior to RT-PCR

1. Set up the DNase digestion reaction as follows:

RNA in water or TE buffer	1–8µl
RQ1 RNase-Free DNase 10X Reaction Buffer	1µl
RQ1 RNase-Free DNase (see Note 1)	1u/µg RNA
Nuclease-free water to a final volume of	10µl

2. Incubate at 37°C for 30 minutes.

   Note: If analyzing RNA samples by gel electrophoresis, perform a phenol:chloroform extraction and ethanol precipitation before loading the samples on the gel, because salts in the RQ1 DNase Reaction Buffer and Stop Solution cause aberrant migration of RNA on gels. Steps 3 and 4 may be omitted if a phenol:chloroform extraction is performed.
3. Add 1µl of RQ1 DNase Stop Solution to terminate the reaction.
4. Incubate at 65°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*[a] *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 $MgSO_4$. When adding the DNase-treated RNA to an RT-PCR reaction, carryover of magnesium must be considered. For example, the addition of 1µl of treated RNA to a 50µl RT-PCR reaction will raise the magnesium concentration by 0.2mM, and the addition of 5µl 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 Mg^{2+} concentration increases up to 5–10mM. At a concentration of 1mM Mg^{2+}, RQ1 DNase is expected to be at least fourfold less active than at the optimal Mg^{2+} concentration.
   - For some templates, the yield from the amplification reaction is highly dependent on the Mg^{2+} concentration, and the optimal 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 fourfold 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 allowing dilution of 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.

II. 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 DNase I footprinting.

A. In Vitro 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*[b,c] *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 *Protocols and Application Guide* (2).

C. Transcription Factor DNase I Footprinting

RQ1 RNase-Free DNase is a component of the 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 8.

D. Production of Random Fragments

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

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^{2+} and Mg^{2+} are essential for RQ1 DNase activity.

III. References

1. Moore, S. (1981) Pancreatic DNase In: *The Enzymes*, Volume 14A, P.D. Boyer, Ed., Academic Press, New York, 281.
2. *Protocols and Applications Guide* (1996) Promega Corporation.
3. Cobianchi, F. and Wilson S.H. (1987) *Meth. Enzymol.* **152**, 94–110.
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, 3.12.
8. Ausubel, F.M. (1994) *Current Protocols in Molecular Biology*, John Wiley and Sons, New York, 12.4.

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