Certificate of Analysis

RQ1 RNase-Free DNase (Cat.# M6101):

Contents

Part No.	Name	Size (units)
M610A	RQ1 RNase-Free DNase	1,000
M199A	Stop Solution	1ml
M198A	RQ1 DNase 10X Reaction Buffer	1ml

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

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 RNA integrity 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).

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 $CaCl_2$. See the unit concentration on the Product Information Label.

Biological Source: Bovine pancreas.

Requirement: Ca^{2+} and Mg^{2+} or Mn^{2+} (6).

Inhibitors: EGTA; EDTA (6); salt concentrations >100mM will reduce DNase activity.

RQ1 DNase 10X Reaction Buffer (M198A): 400mM Tris-HCl (pH 8.0), 100mM $MgSO_4$ and 10mM $CaCl_2$.

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

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

Usage Notes:

1. This DNase solution does not contain an RNase inhibitor. Observe caution in handling the product to avoid 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.
3. To inactivate, heat for 10 minutes at 65°C in the presence of Stop Solution.

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 specification is <3% release.

Signed by:

R. Wheeler, Quality Assurance

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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	1u/µg RNA
Nuclease-free water to a final volume of	10µl

   Note: 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. 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 may cause aberrant migration or smearing 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. See the *Access RT-PCR System Technical Bulletin* #TB220 (5).

Note: The RQ1 RNase-Free DNase digestion contains a final concentration of 10mM $MgSO_4$. When adding DNase-treated RNA to an RT-PCR, carryover of magnesium must be considered. For example, adding 1µl of treated RNA to a 50µl RT-PCR will raise the magnesium concentration by 0.2mM, and adding 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 the amplification reaction.

- RQ1 DNase activity increases as 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 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 RNA integrity 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. Refer to the *Riboprobe® in vitro Transcription Systems 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, refer to the *Protocols and Application Guide* (2).

C. Production of Random Fragments

For protocol information on the use of DNase I for random fragmentation of DNA, see reference 7.

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 reduced 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.
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3. Cobianchi, F. and Wilson S.H. (1987) *Meth. Enzymol.* **152**, 94–110.
4. *Riboprobe® in vitro Transcription Systems Technical Manual* #TM016, Promega Corporation.
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6. Ausubel, F.M. (1994) *Current Protocols in Molecular Biology*, John Wiley and Sons, New York, 3.12.
7. 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.

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