

RQ2.0 DNase I

Instructions for Use of Products **A5100 and A5101**

1. DNase Treatment for DNA Template Removal in In Vitro Transcription Reactions

Perform an in vitro transcription (IVT) reaction. For example, using the RiboMAX™ AOF RNA Production System (Cat.# P2000).

The DNA template can be removed by digestion with RQ2.0 DNase I added directly to the IVT reaction, without buffer exchange or dilution. The following is an example of a post-transcription DNase treatment protocol:

1. After performing the IVT reaction, add RQ2.0 DNase I to a concentration of 1 unit per microgram of template DNA and CaCl₂ to a final concentration of 1.5mM.
2. Incubate for 30–60 minutes at 37°C.
3. Add EGTA and EDTA to final concentrations of 15mM and 1mM, respectively, and incubate at room temperature (20–25°C) for 30 minutes.

Note: Under different buffer conditions, the RQ2.0 DNase I amount required to completely digest a given amount of DNA must be empirically determined. Free rNTPs can chelate Mg²⁺, which inhibits DNase activity. Additional enzyme or Mg²⁺ may be required under these conditions.

There are many options for customization of this protocol. Steps can be performed at lower temperatures, including room temperature, but may require longer time for adequate digestion to occur.

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

We recommend making a master mix of the buffer and water in the volume needed for multiple reactions for pipetting accuracy. Alternatively, RQ2.0 DNase I can be diluted 1:10 in water immediately prior to use to aid pipetting. Discard diluted DNase I after use. The following is an example protocol for DNase treatment of RNA:

1. Set up the DNase digestion reaction as follows.

Reagents	Volume
RNA in water or TE buffer	1–8µl
RQ1 DNase 10X Reaction Buffer	1µl
RQ2.0 DNase I (1u/µg RNA)	0.1µl
Nuclease-free water to a final volume of 10µl	__µl

Note: Use 1 unit of RQ2.0 DNase I per microgram of RNA. For smaller amounts of RNA, use 1 unit of RQ2.0 DNase I per reaction.

2. Incubate at 37°C for 30 minutes.
3. Add 1µl of RQ1 DNase Stop Solution to terminate the reaction.
4. Incubate at 65°C for 10 minutes to inactivate the DNase.

Note: If analyzing RNA samples by gel electrophoresis, perform a phenol:chloroform extraction and ethanol precipitation, column-based purification or other purification method before loading the samples on the gel because salts in the RQ1 DNase Reaction Buffer and Stop Solution may cause aberrant migration or RNA smearing on gels. Steps 3 and 4 may be omitted if RNA purification is performed. Lower RNA yields may be observed following purification due to dilution and loss during the process.

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The RQ2.0 DNase I digestion contains a final concentration of 10mM MgSO_4 .

When adding DNase-treated RNA to an RT-PCR, magnesium carryover must be considered and a purification, dilution or alternative digestion buffer may be required. An alternative DNase reaction buffer may be used (such as the RT reaction or PCR buffer) if that buffer contains at least 1mM Mg^{2+} . Alternatively, the RNA sample may be diluted with water prior to RT-PCR, reducing the MgSO_4 concentration.

3. Other Applications

RQ2.0 DNase I can be used in a number of other applications where maintaining RNA integrity is important. These include nick translation and DNase I footprinting.

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