DNase Treatment using RQ1 (Promega)

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RQ1 RNas...

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 RTPCR 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.