

Title	DNAase Treatment		
Protocol Number	MOLBIO20002	Written by	Duha A.
Adapted from	MO Lab and promega	Version	1.1
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Reagents:

- RQ1 RNase-Free DNase (Cat.# M6101)
- RQ1 RNase-Free DNase 10X Reaction Buffer
- RQ1 DNase Stop Solution
- RNAase free water

Protocol:

1. Calculate the amount of μL s of RNA needed to achieve a total of $5\mu\text{g}$ RNA to be cleaned
2. Based on μL of RNA, digestion reaction should follow:

Reagent	Volume (Final volume, $F_v = 10$)	Volume (Final volume, $F_v = 20$)
RNA in water or TE buffer	1-8 μL	9-13 μL
RQ1 RNase-Free DNase 10X Reaction Buffer	1 μL	2 μL
RQ1 RNase-Free DNase	1 unit per μg of RNA	1 unit per μg of RNA
Nuclease-free water	To a final volume of 10 μL	To a final volume of 20 μL

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.

Note: reaction can be scaled up if the amount of RNA exceeds $8\mu\text{L}$

3. Incubate at 37°C for 30 mints
4. Add $1\mu\text{L}$ (for every $10\mu\text{L}$ in the F_v) of RQ1 DNase Stop Solution to terminate the reaction.
5. Incubate at 65°C for 10 minutes to inactivate the DNase.
6. Proceed to clean up the RNA.

Note: check promega's protocol for sources of contamination that could affect RT-PCR