

QuantiNova™ Reverse Transcription Kit

The QuantiNova Reverse Transcription Kit should be stored immediately upon receipt at -30 to -15°C in a constant-temperature freezer.

Further information

- *QuantiNova Reverse Transcription Kit Handbook*: www.qiagen.com/handbooks
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: toll-free 00800-22-44-6000, or www.qiagen.com/contact

Notes before starting

- Set up all reactions on ice to minimize the risk of RNA degradation.
- RNase inhibitor and dNTPs are already included in the kit components. Do not add additional RNase inhibitor or dNTPs.
- The QuantiNova Internal Control RNA (QN IC RNA) is an internal amplification control that is used to test successful reverse transcription/amplification. The QN IC RNA is intended to report instrument or chemistry failures, errors in assay setup and the presence of inhibitors. It is detected as a 200 bp internal control (IC) in the yellow channel on the Rotor-Gene® Q or in the VIC®/HEX dye channel on other real-time PCR instruments, using the QuantiNova IC Probe Assay (cat no. 205813) or the QuantiTect® Primer Assay for SYBR® Green-based detection (cat no. QT02589307). The IC RNA provided in the QuantiNova Reverse Transcription Kit should be used undiluted.
- A mixture of RT primers is included in the Reverse Transcription Mix. The RT primer mix is optimized to provide high cDNA yields for all RNA transcript regions.
- If gDNA removal is not desired, the removal step can be omitted, but all kit buffer components still need to be combined to enable an

efficient RT reaction. To omit gDNA removal, prepare the Reverse-Transcription Master Mix by combining the Reverse Transcription Mix with the Reverse Transcription Enzyme and add this to the gDNA Removal Mix. Finally, add RNA template and optional IC RNA.

- It is recommended to set up the reactions in 200 µl PCR tubes and to use a PCR cycler for the incubation steps.
- The temperature steps can be conveniently set up using the cycling protocol described in Table 3.

1. Thaw template RNA, QuantiNova Internal Control RNA (optional), gDNA Removal Mix and Reverse Transcription Enzyme on ice. Thaw Reverse Transcription Mix and RNase-free water at room temperature (15–25°C). Mix each solution by flicking the tubes. Centrifuge briefly to collect residual liquid from the sides of the tubes and then keep on ice.
2. Prepare the genomic DNA removal reaction on ice according to Table 1. Mix and then keep on ice.

Note: If setting up more than one reaction, prepare a master mix of gDNA Removal Buffer and RNase-free water with a volume 10% greater than required for the total number of reactions. Distribute the appropriate volume of master mix into individual tubes, followed by each RNA sample.

Note: The protocol is for use with 10 pg – 5 µg RNA. If using >5 µg RNA or a template volume exceeding 12 µl, scale up the reaction linearly while keeping the volume for the QN IC RNA constant. For example, if using 10 µg RNA, double the volumes of all reaction components except the QN IC RNA. Do not exceed a final volume of 30 µl for the gDNA removal reaction.

Table 1. Genomic DNA removal reaction components

Component	Volume/reaction
gDNA Removal Mix*	2 μ l
Template RNA, up to 5 μ g [†]	Variable
Internal Control RNA (optional)	1 μ l
RNase-free water	Variable
Total reaction volume	15 μl

*Also contains RNase inhibitor.

[†]This amount corresponds to the entire amount of RNA present, including any rRNA, mRNA, viral RNA and carrier RNA, regardless of the primers used or cDNA analyzed.

3. Incubate for 2 min at 45°C, then place immediately on ice.

Note: Do not incubate at 45°C for longer than 10 min.

4. Prepare the Reverse-transcription Master Mix on ice according to Table 2. Mix and then keep on ice. The Reverse-transcription Master Mix contains all components required for first-strand cDNA synthesis except template RNA.

Note: If setting up more than one reaction, prepare a volume 10% greater than required for the total number of reactions. Distribute the appropriate volume into individual tubes.

Note: If using >5 μ g RNA, scale up the reverse-transcription reaction linearly but keep the QN IC RNA volume constant. For example, if using 10 μ g RNA, double the volumes of all reverse-transcription reaction components while keeping the volume for the QN IC RNA constant. Do not exceed a final volume of 40 μ l.

Table 2. Reverse-transcription reaction components

Component	Volume/reaction
Reverse-transcription Master Mix:	
Reverse Transcription Enzyme	1 μ l
Reverse Transcription Mix*	4 μ l
Template RNA	15 μ l
Entire genomic DNA elimination reaction (step 3)	(added at step 5)
Total reaction volume	20 μl

*Includes Mg^{2+} and dNTPs.

5. Add freshly prepared Reverse-transcription Master Mix to each tube containing template RNA from step 3 (15 µl). Mix and then store on ice.

Note: Turn off the lid heater if using a PCR cycler with a heated lid. The recommended temperatures in Table 3 will not cause vaporization.

Table 3. gDNA elimination and RT temperature protocol

Step	Time	Temperature	Comment
gDNA elimination reaction	2 min	45°C	
	Pause	25°C	Remove samples, place on ice, add RT components
Reverse-transcription reaction			After adding RT components place samples in the cycler again and continue
Annealing step	3 min	25°C	
Reverse-transcription step	10 min	45°C	
Inactivation of reaction	5 min	85°C	

6. Incubate for 3 min at 25°C.

7. Incubate for 10 min at 45°C.

Note: In some rare cases (e.g., if the RT-PCR product is longer than 200 bp or if analyzing RNAs with a very high degree of secondary structure), increasing the incubation time to 20 min may increase cDNA yields.

8. Incubate for 5 min at 85°C to inactivate the Reverse Transcriptase Enzyme.

9. Place the reverse-transcription reactions on ice and proceed directly with real-time PCR. We recommend diluting the reverse-transcription reaction 1:10 in sterile water. This enables the analysis of multiple transcripts from a single reverse-transcription reaction. For long-term storage, store the reverse-transcription reactions at -30 to -15°C.

Note: QuantiNova SYBR Green PCR or QuantiNova Probe PCR Kits are recommended for real-time PCR, especially when using the QN IC RNA. For detailed information on use of the QN IC RNA and interpretation of real-time PCR results, please refer to the quick-start protocol *QuantiNova Internal Control RNA and Assay*.