Quick-Start Protocol March 2016

QuantiTect® Reverse Transcription Kit

The QuantiTect Reverse Transcription Kit (cat. nos. 205310, 205311, 205313 and 205314) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer.

Further information

- *QuantiTect Reverse Transcription Handbook*: **www.qiagen.com/HB-0189**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Dissolve any precipitates in gDNA Wipeout Buffer by vortexing. If necessary, briefly incubate at 37°C until the precipitates dissolve.
- 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.
- RT Primer Mix (supplied) or gene-specific primers (not supplied) should be used. RT Primer Mix is optimized to provide high cDNA yields for all regions of RNA transcripts. For gene-specific primers, we recommend using a final concentration of 0.7 µM.
- Separate denaturation and annealing steps are not necessary before starting the reverse-transcription reaction.
- If using a reaction volume of 200 µl or greater for reverse transcription, make sure the reaction tube is efficiently heated (e.g., if using a heating block, carefully fill each well with a drop of water so that heat can be efficiently transferred from the block to the tube).
- After reverse transcription, the reaction must be inactivated by incubation at 95°C for 3 min.

- If working with RNA for the first time, refer to Appendix A of the *QuantiTect Reverse Transcription Handbook*.
- If you have purchased the QuantiTect Reverse Transcription Kit in order to perform additional reverse-transcription reactions with the FastLane® Cell cDNA Kit, follow the protocol in the *FastLane Cell cDNA Handbook*. Do not follow this protocol.

1. Thaw template RNA on ice. Thaw gDNA Wipeout Buffer, Quantiscript® Reverse Transcriptase, Quantiscript RT Buffer, RT Primer 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 elimination 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 Wipeout Buffer and RNase-free water with a volume 10% greater than that required for the total number of reactions to be performed. Distribute the appropriate volume of master mix into individual tubes, followed by each RNA sample.

   Note: The protocol is for use with 10 pg to 1 µg RNA. If using >1 µg RNA, scale up the reaction linearly. For example, if using 2 µg RNA, double the volumes of all reaction components for a final 28 µl reaction volume.

Table 1. Genomic DNA elimination reaction components

Component	Volume/reaction
gDNA Wipeout Buffer, 7x	2 µl
Template RNA, up to 1 µg*	Variable
RNase-free water	Variable
Total reaction volume	14 µl

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

3. Incubate for 2 min at 42°C, then place immediately on ice.

   Note: Do not incubate at 42°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 of master mix 10% greater than that required for the total number of reactions to be performed. Distribute the appropriate volume into individual tubes.

   Note: If using >1 µg RNA, scale up the reaction linearly. For example, if using 2 µg RNA, double the volumes of all reaction components for a final 40 µl reaction volume.

Table 2. Reverse-transcription reaction components

Component	Volume/reaction
Reverse-transcription master mix	
Quantiscript Reverse Transcriptase*	1 µl
Quantiscript RT Buffer, 5x†‡	4 µl
RT Primer Mix‡	1 µl
Template RNA	
Entire genomic DNA elimination reaction (step 3)	14 µl (added at step 5)
Total reaction volume	20 µl

* Also contains RNase inhibitor.

† Includes Mg^{2+} and dNTPs.

‡ For convenience, premix RT Primer Mix and 5x Quantiscript RT Buffer in a 1:4 ratio if RT Primer Mix will be used routinely for reverse transcription. This premix is stable when stored at –20°C. Use 5 µl of the premix per 20 µl reaction.

5. Add template RNA from step 3 (14 µl) to each tube containing reverse-transcription master mix. Mix and then store on ice.
6. Incubate for 15 min at 42°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 up to 30 min may increase cDNA yields.

7. Incubate for 3 min at 95°C to inactivate Quantiscript Reverse Transcriptase.
8. Place the reverse-transcription reactions on ice and proceed directly with real-time PCR. For long-term storage, store reverse-transcription reactions at –20°C.

   Note: For details on performing real-time PCR after reverse transcription, refer to Appendix C of the *QuantiTect Reverse Transcription Handbook*. For details on appropriate controls, see Appendix D. We recommend using a Rotor-Gene® Kit, QuantiFast® Kit or QuantiTect Kit for real-time PCR.

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.