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cDNA synthesis using the QuantiTect Reverse transcription kit

In 1 collection

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Abstract

This protocol details the synthesis of cDNA.

cDNA synthesis

50m

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 °C 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.
- 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 00:02:00 at 42 °C , then place immediately On ice .

2m

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.
- 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 00:15:00 at 42 °C . 45m

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 00:30:00 may increase cDNA yields.

7 Incubate for 00:03:00 at 95 °C to inactivate Quantiscript Reverse Transcriptase. 3m

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.