Quick-Start Protocol March 2016

Omniscript® Reverse Transcription Kit

The Omniscript RT Kit (cat. nos. 205110, 205111 and 205113) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer.

Further information

- *Omniscript Reverse Transcription Handbook*: **www.qiagen.com/HB-0490**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- The protocol is optimized for use with 50 ng to 2 µg of RNA. 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. For best results with <50 ng RNA, use Sensiscript® Reverse Transcriptase.
- Separate denaturation and annealing steps are generally not necessary. However, for some RNAs with a high degree of secondary structure, a denaturation step may be desired. If so, denature the RNA in RNase-free water before reaction setup: incubate the RNA for 5 min at 65°C, then place immediately on ice. Do not denature the RNA in the reaction mix.
- Set up all reactions on ice to avoid premature cDNA synthesis and to minimize the risk of RNA degradation.
- When using oligo-dT primers, we recommend a primer length of at least 12 nucleotides and a final concentration of 1 µM.
- When using random primers, we recommend a primer length of 9 nucleotides and a final concentration of 10 µM.

- For some transcripts, more sensitive detection in subsequent PCR or real-time PCR is possible if a mixture of oligo-dT primers and random primers is used.
- When using specific primers, the final primer concentration should be 0.1–1.0 µM.

1. Thaw template RNA on ice. Thaw the primer solutions (not supplied), 10x Buffer RT, dNTP Mix and RNase-free water at room temperature (15–25°C). Store on ice immediately after thawing. Mix each solution by vortexing, and centrifuge briefly to collect residual liquid from the sides of the tubes.
2. Dilute RNase inhibitor (not supplied) to a final concentration of 10 units/µl in ice-cold 1x Buffer RT (dilute an aliquot of 10x Buffer RT accordingly using the RNase-free water supplied). Mix carefully by vortexing for no more than 5 s, and centrifuge briefly to collect residual liquid from the sides of the tube.

   Note: Commercially available RNase inhibitor is commonly supplied at 40 units/µl. Dilution makes it easier to pipet small amounts when preparing the master mix in step 3.

   Note: Prepare a fresh dilution of RNase inhibitor. To minimize the amount of RNase inhibitor and Buffer RT used, dilute no more than needed for your current series of reactions.
3. Prepare a master mix according to Table 1. Mix thoroughly and carefully by vortexing for no more than 5 s. Centrifuge briefly to collect residual liquid from the walls of the tube, and store on ice.

   Note: If setting up more than one reaction, prepare a volume of master mix 10% greater than that required for the total number of reverse transcription reactions to be performed.

   Note: If using >2 µg RNA, scale up the reaction linearly to the appropriate volume. Calculate the scale-up factor from 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.

Table 1. Reverse-transcription reaction components

Component	Volume/reaction
Master mix	
10x Buffer RT	2 µl
dNTP Mix (5 mM each dNTP)	2 µl
Oligo dT primer (10 µM)*	2 µl
RNase inhibitor (10 units/µl)†	1 µl
Omniscript Reverse Transcriptase	1 µl
RNase-free water	Variable
Template RNA (50 ng–2 µg‡) (added at step 5)	Variable
Total reaction volume	20 µl

* Not provided. If using specific primers, the final primer concentration should be 0.1–1.0 µM. If using random primers, we recommend a primer length of 9 nucleotides and a final concentration of 10 µM.

† Not provided.

‡ 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.

4. If setting up more than one reverse-transcription reaction, distribute the appropriate volume of master mix into individual reaction tubes. Keep tubes on ice.
5. Add template RNA to the individual tubes containing the master mix. Mix thoroughly and carefully by vortexing for no more than 5 s. Centrifuge briefly to collect residual liquid from the walls of the tubes.
6. Incubate for 60 min at 37°C.
7. Place the reverse-transcription reactions on ice and proceed directly with PCR. For long-term storage, store reverse-transcription reactions at –20°C.

   Note: When performing real-time PCR, no more than 1/10 of the final PCR volume should derive from the finished reverse-transcription reaction. For example, for a 50 µl PCR assay, use ≤5 µl of the finished reverse-transcription reaction.

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