August 2023

Quick-Start Protocol

# Reverse Transcriptase

Reverse Transcriptase (cat. no. RT32-010) is a modified, recombinant form of the Reverse Transcriptase from Moloney Murine Leukemia Virus (M-MuLV) purified from *E. coli*. Reverse Transcriptase synthesizes a complementary DNA strand in the presence of a primer using either RNA (cDNA synthesis) or single-stranded DNA (ssDNA) as a template.

Reverse Transcriptase is shipped on dry ice. All components should be stored at –20°C in a freezer without a defrost cycle.

## Further information

- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

## Notes before starting

- Acquisition of high quality, intact RNA, free of genomic DNA and RNase traces, is vital for the synthesis of full-length cDNA followed by an accurate quantitative analysis (qPCR). The following recommendations for working with RNA should therefore be followed:
  - Maintain aseptic working conditions: use disposable gloves, changing them as frequently, as required; use RNase-free consumables; only work in an area assigned for working with RNA and with equipment designated for that purpose.

- RNA samples should be stored aliquoted at –70°C. Avoid subjecting the samples to repeated freezing and thawing cycles.

- During RT-PCR preparation keep Reverse Transcriptase and **10x RT** Reaction Buffer on ice or in a freezing rack.
- Use an RNase H treatment for reactions sensitive to residue RNA traces in order to increase the sensitivity of RT-qPCR.
- The quantity of cDNA used when preparing PCR or qPCR reactions should not exceed 1/10 of a final reaction volume; e.g., a maximum volume of 2.5 µL of cDNA should be used in a 25 µL reaction.
- The activity of Reverse Transcriptase is inhibited by metal ion chelating agents (e.g., EDTA), inorganic phosphors, pyrophosphates and polyamines.
- **Enzyme inactivation** should be carried out at 85°C for 5 min.

## Protocol for the first strand cDNA synthesis

1. Add all reaction reagents listed below to a sterile nuclease-free tube placed on ice or in a freezing rack (for a larger quantity of samples, preparing Master Mix without an RNA template is recommended). The reagents should be added in the following order:

**Table 1. Reagents to be added and corresponding quantities**

| | Reagent | Quantity |
|---|---|---|
| RNA | Total RNA<br>mRNA | 10 pg – 5 µg<br>10 pg – 500 ng |
| Primer | oligo(dT)$_{12–18}$ primer mix<br>or random hexamers<br>or specific primers | 1 µL (50 µM)<br>1 µL (50–250 ng)<br>1 µL (pmol) |
| Nucleotides | 10 mM dNTP MIX | 1 µL (final conc. 0.5 mM) |
| Water | Nuclease-free water | Fill up to 16 µL |

**Optional**: For denaturation, incubate the sample at 65°C for 5 min, cool on ice, spin briefly, and return to ice. This denaturation step is necessary if GC-rich templates containing secondary structures are used.

2. Add the reagents listed below to the sample in the order tabulated below.

**Table 2. Order of reagents to be added to the sample**

| Reagent | Quantity |
|---|---|
| **10x RT** Reaction Buffer | 2 µL |
| RNase Inhibitor (optional, not provided) | 1 µL (40 U) |
| Reverse Transcriptase | 1 µL (200 U) |
| Total volume | 20 µL |

3. Mix gently and spin briefly.

   **Optional**: Incubate sample at 25°C for 10 min. If random hexamers are used, this step is mandatory.

4. Incubate at 50°C for 30 min.
5. Stop the reaction at 85°C for 5 min and immediately cool the sample on ice.
6. The cDNA obtained is ready for direct use in PCR or qPCR (undiluted or diluted in nuclease-free water or TE buffer) or can be stored at –20°C or –70°C.

## Document Revision History

| Date | Changes |
|---|---|
| August 2023 | Initial release |

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