July 2024

Quick-Start Protocol

Reverse Transcriptase

Reverse Transcriptase (cat. nos. RT32-010 and RT32-050) 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 mRNA	10 pg – 5 µg 10 pg – 500 ng
Primer	oligo(dT)$_{12–18}$ primer mix or random hexamers or specific primers	1 µL (50 µM) 1 µL (50–250 ng) 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
July 2024	Added catalog number RT32-050

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