Thermostable M-MLV Reverse Transcriptase

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Molecular Biology Products
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Product components

Components	Volume (for 25 reactions) Cat. No. E-1000-25	Volume (for 50 reactions) Cat. No. E-1000-50
Thermostable M-MLV Reverse Transcriptase (200 U/µL)	25 µl	50 µl
5x RT buffer	150 µl	300 µl
0.1 M DTT	50 µl	100 µl

Product description

The thermostable M-MLV reverse transcriptase is designed for first-strand cDNA synthesis from short and long RNA molecules. In a 10-min reaction time, RNA templates up to 10 Kb can be easily reverse-transcribed. This thermostable reverse transcriptase is a genetically-engineered thermostable variant of M-MLV reverse transcriptase. The novel mutation sites in this enzyme have greatly enhanced its thermostability, half-life, processivity, and resistance to inhibitors. This product can also be used for reverse transcription of very small amounts of total RNA or mRNA. Optimized reaction buffer is highly compatible with subsequent PCR and quantitative PCR experiments. The first-strand cDNA produced by this enzyme can be easily used in subsequent PCR, qPCR, and also the generation of full-length cDNA libraries. The high reaction temperature (55 °C) for this enzyme allows the use of GC-rich RNA templates with complex secondary structures.

Features

- The enzyme is highly efficient in the reverse transcription of RNA molecules from different tissue/cell/organism sources.
- The high temperature of the reaction ensures the relaxed structure of RNA templates.
- The reverse transcription reaction is conveniently performed in only 10 minutes.

Protocol for the first-strand cDNA synthesis reaction

The protocol exemplified below describes a 20-µl single reverse transcriptase reaction. The reaction volume and components can be increased proportionately. For setting-up reverse transcription reactions for multiple RNA samples or with different priming strategies, the common components can be prepared as a master-mix to minimize pipetting errors.

1. Thaw all components (Template RNA, dNTP mix, DTT, primer, RT reaction buffer, nuclease-free water) on ice.
2. First, prepare the priming reaction on ice according to the following table for a total volume of 14 µl.

Components for priming reaction (tube A)	Volume
Template RNA (10 pg–5 µg total RNA or 10 pg–500 ng mRNA)	up to 11 µL
Random hexamers (50 µM), Oligo-dT primer (50 µM), or Gene-specific reverse primer (2 µM)	1 µl
dNTP mix (10 mM each)	2 µl
Nuclease-free or DEPC-treated water	up to 14 µL

3. Briefly mix and spin the reaction tube.
4. Incubate the tube at 65 °C for 5 minutes, followed by incubation on ice for a minimum of one minute.
5. Add the following components in a new reaction tube on ice.

Components for RT reaction (tube B)	Volume
5x RT reaction buffer	4 µl
0.1 M DTT	1 µl
Thermostable M-MLV Reverse Transcriptase (200 U/µL)	1 µl

6. Briefly mix the contents of tube B.
7. Add the contents of tube B to tube A.
8. If priming is performed with random hexamers, incubate the mixture at 23 °C for 10 minutes. Otherwise, when using oligo-dT or gene-specific primers, continue the protocol from step 9.
9. Incubate the reaction mixture at 55 °C for 10 minutes.
10. (Optional) The enzyme may be inactivated at 80 °C for 10 minutes.
11. The reverse transcription products can be immediately used in the subsequent PCR/qPCR reactions, or stored at –20 °C. ■

Expiry date: 12 month from the date of manufacture
Date of manufacture: