Maxima Reverse Transcriptase

Catalog Number EP0741, EP0742, EP0743

Pub. No. MAN0012044 **Rev.** D.00

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support.**

Contents and storage

Cat No.	Contents	Amount	Storage
EP0741	Maxima Reverse Transcriptase, 200 U/µL	2000 U	-25 °C to -15 °C
	5X RT Buffer*	1 mL	
EP0742	Maxima Reverse Transcriptase, 200 U/µL	10000 U	
	5X RT Buffer*	2 x 1 mL	
EP0743	Maxima Reverse Transcriptase, 200 U/µL	4 x 10000 U	
	5X RT Buffer*	4 x 1 mL	

*5X RT Buffer is also available separately (#B91)

Description

Thermo Scientific™ Maxima™ Reverse Transcriptase (RT) is a novel reverse transcription enzyme that was developed by Thermo Scientific through *in vitro* evolution of M-MuLV RT. The enzyme possesses an RNA and DNA-dependent polymerase activity as well as RNase H activity.

Features

- High yields of full-length cDNA up to 20 kb
- Active up to 65 °C
- Thermostable – 90% active after incubation at 50 °C for 60 minutes in a reaction mixture
- High sensitivity - reproducible cDNA synthesis from a wide range of starting total RNA amounts (1 pg - 5 µg)
- Efficient – complete cDNA synthesis in 15-30 minutes
- Incorporates modified nucleotides

Applications

- First strand cDNA synthesis
- RT-PCR
- RT-qPCR
- DNA labeling
- Primer extension

Source

E.coli cells carrying an engineered *pol* gene fragment of Moloney Murine Leukemia Virus.

Definition of Activity Unit

One unit of the enzyme incorporates 1 nmol of dTMP into a polynucleotide fraction in 10 min at 37 °C.

Storage Buffer

The enzyme is supplied in: 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 1 mM EDTA, 5 mM DTT, detergent and 50% (v/v) glycerol.

5X RT Buffer

250 mM Tris-HCl (pH 8.3 at 25 °C), 375 mM KCl, 15 mM $MgCl_2$, 50 mM DTT.

Inhibition and Inactivation

- Inhibitors: metal chelators, inorganic phosphate, pyrophosphate, and polyamines.
- Inactivated by heating at 85 °C for 5 min.

Protocol for First Strand cDNA Synthesis

The following protocol is optimized to generate first-strand cDNA for use in two-step RT-PCR.
Mix and briefly centrifuge all components after thawing, keep on ice.

1. Add reaction components into a sterile, nuclease-free tube on ice in the indicated order:

Components	Volume
Template RNA: total RNA poly(A) RNA specific RNA	 1 pg - 5 µg 0.1 pg - 500 ng 0.01 pg - 500 ng
Primer: Oligo(dT)$_{18}$ (#SO131) Random hexamer (#SO142) gene-specific primer	 1 µL (100 pmol) 1 µL (100 pmol) 15-20 pmol
dNTP Mix, 10 mM each (#R0191)	1 µL (0.5 mM final concentration)
Water, nuclease-free	to 14.5 µL

2. **Optional:** If the RNA template is GC-rich or is known to contain secondary structures, mix gently, centrifuge briefly and incubate at 65 °C for 5 min. Chill on ice, briefly centrifuge again and place on ice.
3. Add the following components in the indicated order:

Components	Volume
5X RT Buffer	4 µL
Thermo Scientific™ RiboLock RNase Inhibitor (#EO0381)	0.5 µL (20 U)
Maxima Reverse Transcriptase	1 µL (200 U)
Total volume	**20 µL**

Mix gently and centrifuge briefly.

4. Incubate:

- if an oligo(dT)$_{18}$ primer or gene-specific primer is used, incubate for 30 min at 50 °C.
- if a random hexamer primer is used, incubate for 10 min at 25 °C followed by 30 min at 50°C.
  For transcription of GC-rich RNA, the reaction temperature can be increased to 65 °C.

5. Terminate the reaction by heating at 85 °C for 5 minutes.

Note

- The reverse transcription reaction product can be used directly in PCR or qPCR, or stored at -20 °C for up to one week. For longer storage, -70 °C is recommended. Avoid freeze/thaw cycles of the cDNA.
- Use 2 µL of the reaction mix to perform PCR in a 50 µL volume.

Recommendations for two-step RT-qPCR

- Priming: use a mix of oligo (dT)$_{18}$ and random primers 25 pmol each per 20 µL reaction.
- Incubation: 10 min at 25 °C followed by 15 min at 50 °C.

Recommendations for long RT-PCR (>5 kb)

- Priming: oligo (dT)$_{18}$ or gene specific primer should be used.
- Use 20 U of Maxima Reverse Transcriptase per reaction. 1X RT buffer can be used to dilute the enzyme just prior to reaction.
- Incubation: 30 min at 50 °C.

Revision history: Pub. No. MAN0012044

Revision	Date	Description
D.00	2024-01-18	Revized user guide template, removed COA content and updated storage buffer composition.

Limited product warranty

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