Maxima H Minus Reverse Transcriptase

Catalog Number EP0751, EP0752, EP0753

Pub. No. MAN0012047 **Rev.** C.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
EP0751	Maxima H Minus Reverse Transcriptase, 200 U/µL	2000 U	-25 °C to -15 °C
	5X RT Buffer*	1 mL	
EP0752	Maxima H Minus Reverse Transcriptase, 200 U/µL	10000 U	
	5X RT Buffer*	2 x 1 mL	
EP0753	Maxima H Minus 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™ H Minus Reverse Transcriptase (RT) is a novel RT enzyme that was developed through *in vitro* evolution of M-MuLV RT. The enzyme possesses an RNA and DNA-dependent polymerase activity but lacks RNase H activity. The engineered enzyme features dramatically improved thermostability, processivity and an increased synthesis rate compared to wild type M-MuLV RT. Eliminated RNase H activity ensures high yields of full length cDNA products up to 20 kb.

Features

- Thermostable – 90% active after incubation at 50 °C for 60 min in a reaction mixture
- Active up to 65 °C
- RNase H minus - high yields of cDNA up to 20 kb
- High sensitivity - reproducible cDNA synthesis from a wide range of starting total RNA amounts (1 pg – 5 µg)
- Efficient – completes cDNA synthesis in 15-30 minutes
- Increased resistance to common reaction inhibitors
- Incorporates modified nucleotides

Applications

- First strand cDNA synthesis for RT-PCR and RT-qPCR.
- Synthesis of full length cDNA for cloning and expression.
- Generation of labeled cDNA probes for microarrays.
- Analysis of RNA by 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 is a general protocol for first-strand cDNA synthesis:
Mix and briefly centrifuge all reagents 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 reaction components in the indicated order:

Components	Volume
5X RT Buffer	4 µL
Thermo Scientific™ RiboLock RNase Inhibitor (#EO0381)	0.5 µL (20 U)
Maxima H Minus Reverse Transcriptase	50 - 200 U*
Total volume	**20 µL**

*To generate highest absolute amounts of RT reaction products (in applications such as synthesis of labelling probes) use 200 U of enzyme per reaction. For downstream applications, such as PCR or qPCR optimize enzyme amounts within a range of 50 U to 200 U.

Mix gently and centrifuge briefly.

4. Incubate:

- if an oligo(dT)$_{18}$ primer or gene-specific primer is used, incubate for 15-30 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 cDNA reaction in 50 µL of PCR mix.

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.
- Enzyme amount: use 20 U of Maxima H Minus 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. MAN0012047

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

Limited product warranty

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