



## PRODUCT INFORMATION

# Thermo Scientific Maxima H Minus Reverse Transcriptase

Pub. No. MAN0012047

Rev. Date 17 June 2016 (Rev. B.00)

Lot \_\_\_                      Expiry Date \_\_\_

Components	#EP0751	#EP0752	#EP0753
Maxima H Minus Reverse Transcriptase, 200 U/ $\mu$ L	2000 U	10000 U	4 $\times$ 10000 U
5X RT Buffer	1 mL	2 $\times$ 1 mL	4 $\times$ 1 mL

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

**Store at -20 °C**

[www.thermofisher.com](http://www.thermofisher.com)

**For Research Use Only.** Not for use in diagnostic procedures.

## 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  $\mu$ g).
- 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, 0.1% (v/v) Triton X-100 and 50% (v/v) glycerol.

**5X RT Buffer**

250 mM Tris-HCl (pH 8.3 at 25 °C), 375 mM KCl, 15 mM MgCl<sub>2</sub>, 50 mM DTT.

**Inhibition and Inactivation**

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

**CERTIFICATE OF ANALYSIS****Endodeoxyribonuclease Assay**

No detectable degradation was observed after incubation of supercoiled plasmid DNA with Maxima H Minus Reverse Transcriptase.

**Ribonuclease Assay**

No detectable degradation was observed after incubation of [3H]-RNA with Maxima H Minus Reverse Transcriptase.

**Labeled Oligonucleotide (LO) Assay**

No detectable degradation after incubation of single-stranded or double-stranded radiolabeled oligonucleotides with Maxima H Minus Reverse Transcriptase.

**Functional Assay**

Maxima H Minus Reverse Transcriptase was functionally tested in 1.3 kb first strand cDNA synthesis.

Quality authorized by:

 Jurgita Zilinskiene

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## 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:

Template RNA	total RNA <i>or</i> poly(A) RNA <i>or</i> specific RNA	1 pg – 5 µg  0.1 pg – 500 ng  0.01 pg - 500 ng
Primer	Oligo(dT) <sub>18</sub> (#SO131) <i>or</i> Random Hexamer (#SO142) <i>or</i> 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:

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

\*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)<sub>18</sub> 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)<sub>18</sub> 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)<sub>18</sub> 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.

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