Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific Maxima H Minus Reverse Transcriptase

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Lot __ **Expiry Date __**

Components	#EP0751	#EP0752	#EP0753
Maxima H Minus Reverse Transcriptase, 200 U/µL	2000 U	10000 U	4 × 10000 U
5X RT Buffer	1 mL	2 × 1 mL	4 × 1 mL

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

Store at -20 °C

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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 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, 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 $MgCl_2$, 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 *or* poly(A) RNA *or* specific RNA	1 pg – 5 µg 0.1 pg – 500 ng 0.01 pg - 500 ng
Primer	Oligo(dT)$_{18}$ (#SO131) *or* Random Hexamer (#SO142) *or* 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
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.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively *for research purposes and in vitro use only.* The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to www.thermofisher.com for Material Safety Data Sheet of the product.