PRODUCT INFORMATION

Thermo Scientific Maxima H Minus Reverse Transcriptase

#EP0751 2000 U

Lot: __ Expiry Date: __

Concentration: 200 U/µL

Supplied with: 1 mL of 5X RT Buffer

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

Store at -20°C

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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 (adsorbed on DE-81) in 10 min at 37°C.

Enzyme activity is assayed in the following mixture:
50 mM Tris-HCl (pH 8.3), 4 mM $MgCl_2$, 10 mM DTT,
50 mM KCl, 0.5 mM dTTP, 0.4 MBq/mL [^{3}H]-dTTP,
0.4 mM poly(A)·oligo $(dT)_{12\text{-}18}$.

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 conversion of covalently closed circular DNA to nicked DNA was detected after incubation of 2000 units of the enzyme with 1 µg of pUC19 DNA for 4 hours at 37°C.

Ribonuclease Assay

No contaminating RNase activity was detected after incubation of 200 units of the enzyme with 1 µg of [^{3}H]-RNA for 4 hours at 37°C.

Labeled Oligonucleotide (LO) Assay

No degradation of single-stranded and double-stranded labelled oligonucleotide was observed after incubation with 400 units of the enzyme for 4 hours at 37°C.

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.

Note

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