



PRODUCT INFORMATION

Thermo Scientific Maxima Reverse Transcriptase

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Lot: ___ Expiry Date: ___

Store at -20 °C

Components	#EP0741	#EP0742	#EP0743
Maxima 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)

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

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.
- Thermostabile – 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, 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 MgCl₂, 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 Reverse Transcriptase

Ribonuclease Assay

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

Labeled Oligonucleotide (LO) Assay

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

Functional Assay

Maxima Reverse Transcriptase was tested in synthesis of 1.3 kb first strand cDNA.

Quality authorized by:



Jurgita Zilinskiene

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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 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	1 pg - 5 µg
	<i>or</i> poly(A) RNA	0.1 pg - 500 ng
	<i>or</i> specific RNA	0.01 pg - 500 ng
Primer	Oligo(dT) ₁₈ (#SO131)	1 µL (100 pmol)
	<i>or</i> Random Hexamer (#SO142)	1 µL (100 pmol)
	<i>or</i> gene-specific primer	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 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.

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