Fermentas
LIFE SCIENCES

CERTIFICATE OF ANALYSIS

Maxima®
Reverse Transcriptase

#EP0742 10000 u

Lot: __ **Expiry Date: __**

Concentration: 200 u/µl
Supplied with: 1 ml of 5X RT Buffer

Store at -20°C

Description

Maxima® Reverse Transcriptase (RT) is a novel reverse transcription enzyme that was developed by Fermentas 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 60°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 (10 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 (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 $(dT)_{12-18}$.

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.

QUALITY CONTROL ASSAY DATA

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 2000 units of enzyme with 1 µg of pUC19 DNA in 50 µl of buffer for 4 hours at 37°C.

Labeled Oligonucleotide (LO) Assay

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

Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 200 units of enzyme with 1 µg of [^{3}H]-RNA in 50 µl of buffer for 4 hours at 37°C.

Functional Assay

Maxima® Reverse Transcriptase was tested for use in the first strand cDNA synthesis.

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 *or* poly(A) RNA *or* specific RNA	10 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
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 60°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.
- 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.

Note

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.fermentas.com for Material Safety Data Sheet of the product.