PRODUCT INSERT

GeneSure M-MuLV Reverse Transcriptase

Store at -20°C

Cat. No.	Pack Size
PGM044	10000 U

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

Description
GeneSure M-MuLV Reverse Transcriptase (RT) is a genetically modified M-MuLV RT. It differs from wildtype M-MuLV RT by its structure, catalytic properties and in the optimum activity temperature. The enzyme possesses RNA-dependent and DNA-dependent polymerase activity and a RNase H activity specific to RNA in RNA-DNA hybrids which is significantly lower than that of Avian Myeloblastis Virus (AMV) reverse transcriptase.
GeneSure M-MuLV Reverse Transcriptase activity is optimal at 42°C (active up to 50°C). The enzyme is capable of first strand cDNA synthesis up to 13 kb. The enzyme incorporates modified nucleotides.

Applications
- First strand cDNA synthesis for RT-PC R and real-time RT-PCR
- Synthesis of cDNA for cloning and expression.
- Generation of labeled cDNA probes for microarrays.
- DNA labeling.
- Analysis of RNA by primer extension.

Source
E.coli cells with a cloned fragment of the pol gene encoding Moloney Murine Leukemia Virus reverse transcriptase.

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 MgCl2, 10 mM DTT,
50 mM KCl, 0.5 mM dTTP, 0.4 MBq/ml [3H]-dTTP,
0.4 mM polyA·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 Reaction Buffer
250 mM Tris-HCl (pH 8.3 at 25°C), 250 mM KCl,
20 mM MgCl2, 50 mM DTT.

Inhibition and Inactivation
- Inhibitors: metal chelators, inorganic phosphate, pyrophosphate and polyamines .
- Inactivated by heating at 70°C for 10 min.

Note
GeneSure RT has much lower RNase H activity than Avian Myeloblastosis Virus (AMV) reverse transcriptase.

Endodeoxyribonuclease Assay
No conversion of covalently closed circular DNA to nicked DNA was detected after incubation of 2000 units of GeneSure M-MuLV Reverse Transcriptase 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 GeneSure M-MuLV Reverse Transcriptase with 1 µg of [3H]-RNA for 4 hours at 37°C.

Labeled Oligonucleotide (LO) Assay
No degradation of single-stranded and double-stranded labeled oligonucleotide was observed after incubation with 400 units of GeneSure M-MuLV Reverse Transcriptase for 4 hours at 37°C.

Functional Assay
GeneSure M-MuLV Reverse Transcriptase was tested in synthesis of 1.3 kb first strand cDNA.

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 components after thawing, keep on ice.

1. Add into sterile, nuclease-free tube on ice in the indicated order:

Template RNA	total RNA, or poly(A) RNA, or specific RNA	0.1ng -5ug 10pg -500pg 0.01ng -0.5ug
Primer	Oligi(dt), or Random hexamer, or Gene-specific primer	0.5ug (100pmol) 0.2ug (100pmol) 15-20 pmol
DEPC-treated water		to 12.5ul

2. Optional: If 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 and place on ice.

3. Add the following components in the indicated order:

5X Reaction Buffer	4 µl
RNase Inhibitor	0.5 µl (20 u)
dNTP Mix, 10 mM each	2 µl (1 mM final concentration)
GeneSure H Minus M-MuLV R T	1 µl (200 U)
Total volume	**20 µl**

Mix gently and centrifuge briefly.

4. If oligo(dT)18 primer or gene-specific primer is used, incubate 60 min at 42°C. If random hexamer primer is used, incubate 10 min at 25°C followed by 60 min at 42°C. For transcription of GC rich RNA reaction temperature can be increased to 45°C.
5. Terminate the reaction by heating at 70°C for 10 min. Do not heat-inactivate enzyme prior to analysis of long cDNA to avoid cleavage.

Note
- The reverse transcription reaction product can be directly used in PCR or stored at -20°C.
- Use 2 µl of the reaction mix to perform PCR in 50 µl volume.