PRODUCT INFORMATION

Thermo Scientific RevertAid H Minus Reverse Transcriptase

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Lot: _ **Expiry Date:** _

Components	#EP0451	#EP0452
RevertAid H Minus Reverse Transcriptase, 200 U/µL	10000 U	5 × 10000 U
5X Reaction Buffer	1 mL	5 × 1 mL

Store at -20 °C

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Description

Thermo Scientific™ RevertAid™ H Minus Reverse Transcriptase (RT) is a genetically modified M-MuLV RT. The enzyme possesses RNA-dependent and DNA dependent polymerase activity, but lacks RNase H activity due to point mutation in the RNase H domain (1, 2). RevertAid H Minus Reverse Transcriptase does not degrade RNA in RNA-DNA hybrids during synthesis of the first strand cDNA and therefore high yields of full-length cDNA from long templates are obtained. RevertAid H Minus Reverse Transcriptase maintains activity over a wide temperature range (42-55 °C) and is capable of full-length first strand cDNA synthesis up to 13 kb. The enzyme incorporates modified nucleotides.

Applications

- First strand cDNA synthesis for RT-PCR and real-time RT-qPCR (3, 4, 5), *see* protocol on back page.
- Reverse transcription at elevated temperatures to reduce effects of secondary structure.
- Synthesis of cDNA for cloning and expression.
- Generation of labeled cDNA probes for microarrays (6).
- DNA labeling (3).
- Analysis of RNA by primer extension (3).

Source

E.coli cells carrying a cloned fragment of the mutated *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 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 Reaction Buffer

250 mM Tris-HCl (pH 8.3 at 25 °C), 250 mM KCl, 20 mM $MgCl_2$, 50 mM DTT.

Inhibition and Inactivation

- Inhibitors: metal chelators, inorganic phosphate, pyrophosphate and polyamines (2).
- Inactivated by heating at 70 °C for 10 min.

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No detectable degradation was observed after incubation of supercoiled plasmid DNA with RevertAid H Minus Reverse Transcriptase.

Ribonuclease Assay

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

Labeled Oligonucleotide (LO) Assay

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

Functional Assay

RevertAid H Minus 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 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	0.1 ng-5 µg 10 pg-500 ng 0.01 pg-0.5 µg
Primer	Oligo(dT)$_{18}$ (#SO131) *or* Random hexamer (#SO142) *or* gene-specific primer	0.5 µg (100 pmol) 0.2 µg (100 pmol) 15-20 pmol
DEPC-treated water (#R0601)		to 12.5 µL

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
Thermo Scientific™ RiboLock™ RNase Inhibitor (#EO0381)	0.5 µL (20 U)
dNTP Mix, 10 mM each (#R0191)	2 µL (1 mM final concentration)
RevertAid H Minus Reverse Transcriptase	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 55 °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.

References

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