



PRODUCT INFORMATION

Thermo Scientific RevertAid Reverse Transcriptase

Pub. No. MAN0012757

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Lot: __ **Expiry Date:** __

| Components | #EP0441 | #EP0442 |
|---|---------|--------------------|
| RevertAid Reverse Transcriptase, 200 U/ μ L | 10000 U | 5 \times 10000 U |
| 5X Reaction Buffer | 1 mL | 5 \times 1 mL |

Store at -20 °C

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Description

Thermo Scientific RevertAid 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 (1,2).

RevertAid™ 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-PCR and real-time RT-PCR, see protocol on back page.
- Synthesis of cDNA for cloning and expression.
- Generation of labeled cDNA probes for microarrays.
- DNA labeling (3).
- Analysis of RNA by primer extension (3).

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 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 MgCl₂, 50 mM DTT.

Inhibition and Inactivation

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

Note

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

CERTIFICATE OF ANALYSIS**Endodeoxyribonuclease Assay**

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

Ribonuclease Assay

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

Labeled Oligonucleotide (LO) Assay

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

Functional Assay

RevertAid 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 | 0.1 ng-5 µg |
| | <i>or</i> poly(A) RNA | 10 pg-500 ng |
| | <i>or</i> specific RNA | 0.01 pg-0.5 µg |
| Primer | Oligo(dT) ₁₈ (#SO131) | 0.5 µg (100 pmol) |
| | <i>or</i> Random hexamer (#SO142) | 0.2 µg (100 pmol) |
| | <i>or</i> gene-specific primer | 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 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 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.

References

1. Verma, I.M., Reverse transcriptase, The Enzymes (Boyer, P.D., ed), Academic Press Inc., vol. 14, 87-103, 1981.
2. Gerard, G.F. and D'Alessio, J.M., Methods in Molecular Biology, 16, Humana Press, Totowa, N.J., 73-93, 1993.
3. Sambrook, J., Russell, D.W., Molecular Cloning: A Laboratory Manual, the third edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001.

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