Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific RevertAid Reverse Transcriptase

Pub. No. MAN0012757
Rev. Date 17 June 2016 (Rev. B.00)

Lot: _ **Expiry Date: _**

Components	#EP0441	#EP0442
RevertAid Reverse Transcriptase, 200 U/µL	10000 U	5 × 10000 U
5X Reaction Buffer	1 mL	5 × 1 mL

Store at -20 °C

www.thermofisher.com

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

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 $MgCl_2$, 50 mM DTT.

Inhibition and Inactivation

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

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

(continued on back page)

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 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.

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