

PRODUCT INFORMATION

Thermo Scientific RevertAid Reverse Transcriptase

#EP0441 **10000 u**

Lot: **Expiry Date:**

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

Store at -20°C

In total 2 vials.

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 (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 (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 conversion of covalently closed circular DNA to nicked DNA was detected after incubation of 2000 units of RevertAid 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 RevertAid 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 RevertAid Reverse Transcriptase for 4 hours at 37°C.

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 (#S0131)	0.5 µg (100 pmol)
	<i>or</i> Random hexamer (#S0142)	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 (#E00381)	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.thermoscientific.com/fermentas for Material Safety Data Sheet of the product.

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