

CERTIFICATE OF ANALYSIS

**RevertAid™ H Minus M-MuLV
Reverse Transcriptase**

#EP0452 5 x 10000 u

Lot: **Expiry Date:**

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

Store at -20°C

In total 10 vials.

Description

RevertAid™ H Minus M-MuLV Reverse Transcriptase (RT) is a genetically modified M-MuLV RT. It differs from the M-MuLV RT by its structural and catalytic properties. The enzyme possesses an RNA- and DNA-dependent polymerase activity, but lacks ribonuclease H activity specific to RNA in RNA-DNA hybrids (1, 2). RNase H activity is eliminated by a point mutation in the RNase H domain of M-MuLV RT (1, 2).

Source

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

Features

- High yields of full-length first strand cDNA up to 13 kb.
- Optimum of activity at 42-45°C.
- Active up to 55°C.
- Incorporates modified nucleotides (e.g. Cy3-, Cy5-, rhodamine-, aminoallyl-, fluorescein-labeled nucleotides).

Applications

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

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), 6 mM MgCl₂, 10 mM DTT, 40 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% Triton X-100 and 50% 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.

QUALITY CONTROL ASSAY DATA

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 2000 units of enzyme with 1 µg of pBR322 DNA in 50 µl of reaction buffer for 4 hours at 37°C.

Exodeoxyribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 2000 units of enzyme with 1 µg of sonicated *E.coli* [³H]-DNA in 50 µl of reaction buffer for 4 hours at 37°C.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of a single-stranded and double-stranded labeled oligonucleotide was observed after incubation with 2000 units of enzyme for 4 hours at 37°C.

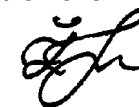
Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 2000 units of enzyme with 1 µg of [³H]-RNA in 50 µl of reaction buffer for 4 hours at 37°C.

Functional Assay

RevertAid™ H Minus M-MuLV Reverse Transcriptase was tested for use in the first strand cDNA synthesis and RT-PCR.

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.

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

Template RNA	total RNA	10 ng-5 µg
	<i>or</i> poly(A) RNA	1-500 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 (#S0141)	0.2 µg (100 pmol)
	<i>or</i> gene-specific primer	15-20 pmol
DEPC-treated water (#R0601)		to 12.5 µl

- ❷ **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.

- ❸ Add the following components in the indicated order:

5X reaction buffer	4 µl
RiboLock™ RNase Inhibitor (#E00381)	0.5 µl (20 u)
dNTP Mix, 10 mM each (#R0191)	2 µl (1 mM final concentration)
RevertAid™ H-Minus M-MuLV Reverse Transcriptase	1 µl (200 u)
Total volume	20 µl

Mix gently and centrifuge briefly.

- ❹ 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.
- ❺ 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.
4. Schmidt, A., Su, Y.H., et al., UPS1 and UPS2 from Arabidopsis Mediate High Affinity Transport of Uracil and 5-Fluorouracil, J. Biol. Chem., 279, 44817-44824, 2004.
5. Papavinasasundaram, K.G., et al., Deletion of the Mycobacterium tuberculosis pknH Gene Confers a Higher Bacillary Load during the Chronic Phase of Infection in BALB/c Mice, J. Bacteriol, 187, 5751-5760, 2005.
6. Turk, R., et al., Gene expression variation between mouse inbred strains, BMC Genomics, 5:57, 2004.

DE-81 – Whatman anion exchange chromatography paper having diethylaminoethyl functional groups.

Whatman is a registered trademark of Whatman Ltd.

Triton X-100 is a registered trademark of Rohm & Haas, Inc.

Note. RevertAid™ H Minus M-MuLV Reverse Transcriptase not available in the USA.

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

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Related Products

- Oligo(dT)₁₈ Primer #S0131
- Random Hexamer Primer #S0141
- dNTP Mix, 10 mM each #R0191
- dNTP Mix, 25 mM each #R1121
- Aminoallyl-dUTP #R0091
- Biotin-11-dUTP #R0081
- Fluorescein-12-dUTP* #R0101
- RiboLock™ RNase Inhibitor #E00381
- DreamTaq™ DNA Polymerase #EP0701
- Taq DNA Polymerase (recombinant) #EP0401
- Pfu DNA polymerase (native)* #EP0571
- Pfu DNA polymerase (recombinant)* #EP0501
- PCR Master Mix (2X) #K0171
- Maxima™ Hot Start Taq DNA Polymerase #EP0601
- TrueStart™ Taq DNA Polymerase #EP0611
- PyroStart™ Fast PCR Master Mix (2X) #K0211
- High Fidelity PCR Enzyme Mix* #K0191
- Long PCR Enzyme Mix* #K0181
- DNase I, RNase-free #EN0521
- RNase H, *E. coli* #EN0201
- DNA Polymerase I, *E. coli* #EP0041
- DEPC-treated Water #R0601
- Water, nuclease-free #R0581

* not available in the USA