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LIFE SCIENCES

CERTIFICATE OF ANALYSIS

RevertAid™ Reverse Transcriptase

#EP0442 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.

ISO 9001 ISO 14001
www.fermentas.com

Description

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 $MgCl_2$, 10 mM DTT, 50 mM KCl, 0.5 mM dTTP, 0.4 MBq/ml [^{3}H]-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 $MgCl_2$, 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.

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 pUC19 DNA in 50 µl of (25 mM Tris-HCl, 5 mM $MgCl_2$, 5 mM NaCl (pH 8.0)) 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 400 units of enzyme for 4 hours at 37°C.

Ribonuclease Assay

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

Functional Assay

200 units of RevertAid™ Reverse Transcriptase were incubated with 1.1 kb RNA as a template and oligo $(dT)_{18}$ as a primer. The reaction product was analyzed on 1.4% alkaline agarose gel. After ethidium bromide staining a 1.1 kb cDNA band was visualized as a predominant band.

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	100 ng-5 µg 10-500 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
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.