Print

Reverse Transcriptase, M-MuLV

From *Escherichia coli* HB 101 pB6B15.23
Deoxynucleoside-triphosphate: DNA deoxynucleotidyl-transferase (RNA-directed), *EC 2.7.7.49*

Cat. No. 11 062 603 001

500 U

Version 19
Content version: April 2011

Store at −15 to −25°C

Product Overview

Supplied cDNA Synthesis Buffer (First Strand)

Incubation buffer for the M-MuLV reverse transcriptase:
5× conc.: 250 mM Tris-HCl, 200 mM KCl, 30 mM $MgCl_2$, 50 mM dithioerythritol, pH 8.3 (+37 °C).

Volume Activity

20×10^3 U/ml. One unit is the enzyme activity which incorporates 1.0 nmol TMP into an acid-insoluble product within 10 min at +37 °C with poly(A) x $d(T)_{15}$ as substrate (1).

Specific Activity

$>40 \times 10^3$ U/mg.

Storage Buffer

50 mM Tris-HCl, pH 8.4 (+4 °C), 10 mM dithioerythritol,100 mM NaCl, 0.05% Polydocanol (v/v), 1 mM EDTA, 50% glycerol (v/v).

Description

Reverse Transcriptase, M-MuLV is produced as a cloned enzyme resulting from the fusion of the *E. coli* trpE gene with the central region of the M-MuLV pol gene. The enzyme is expressed in *E. coli* as a single polypeptide with a molecular weight of 71 kDa (2) as determined by SDS-polyacrylamide gel electrophoresis. The cloned enzyme retains full reverse transcriptase and RNase H activities (2).

Application

Reverse transcriptase is used for:

- *In vitro* synthesis of cDNA from specific RNA sequences for the preparation of cDNA libraries (3).
- Synthesis of first strand cDNA for use in subsequent amplification reactions.

The cDNA products are used to analyze structure, organization, and expression of prokaryotic and eukaryotic genes. Comparison between cDNA and genomic DNA sequences elucidates intervening sequences, splicing events, and genomic rearrangements in eukaryotic genes. Reverse transcriptase is used in dideoxy sequencing reactions in place of Klenow enzyme; reverse transcriptase will often synthesize through GC-rich regions where Klenow enzyme is hindered.

Storage/ Stability

The undiluted enzyme solution is stable at −15 to −25°C until the expiration date printed on the label.

Standard Assay for First Strand cDNA Synthesis

Additional Reagents Required

- Primer for cDNA Synthesis $p(dT)_{15}$*
- Set of dATP, dCTP, dGTP, dTTP, PCR grade*
- RNase Inhibitor*

Protocol

1. Pipet in a microfuge tube on ice, mix, and make up to a final volume of 20 µl:

Reagent	Volume
5× Incubation buffer (= 1× end concentration)	4 µl
2 µg poly(A)$^+$ RNA (*e.g.,* neo pA approximately 1 kb)	× µl
20 µCi [α-^{32} P]dCTP (3,000 Ci/mMol = 110 TBq/mMol)	× µl
40 mA_{260} U primer $p(dT)_{15}$	× µl
20 nMol each of dATP, dCTP, dGTP and dTTP	× µl
25 U RNase inhibitor	× µl
40 U M-MuLV, Reverse Transcriptase	× µl

2. Incubate at +37°C for 60 min.

   The resulting first strand cDNA can be readily used for second strand synthesis, hybridization, or amplification by the PCR. A protocol for the preparation of second strand cDNA is supplied with the cDNA Synthesis Kit (Cat. No. 11 117 831 001) or (4).

Results

The incorporation rate is $> 2 \times 10^5$ cpm. This corresponds to 15 - 30% conversion to cDNA, and depends on the quality of the template RNA. The cDNA products obtained are >80% “full length”.

Quality Control

Absence of Endonucleases

1 µg MWM II DNA is incubated for 16 hours at +37°C with increasing amounts of reverse transcriptase. Incubation with up to 150 U Reverse Transcriptase, M-MuLV does not change the banding pattern obtained using gel electrophoresis.

Absence of Nicking Activity

1 µg supercoiled pBR322 DNA is incubated for 16 hours at +37°C with increasing amounts of reverse transcriptase. Incubation with up to 150 U Reverse Transcriptase, M-MuLV does not show relaxation of supercoiled pBR322 DNA.

Absence of Ribonucleases

5 µg of MS2 RNA are incubated with different amounts of reverse transcriptase for 4 hours at +37°C. Incubation with up to 150 U Reverse Transcriptase, M-MuLV does not show degradation of MS2 RNA.

Absence of Exonuclease Activity

Approximately 5 µg [^{3}H] labeled DNA are incubated with 3 µl reverse transcriptase, M-MuLV for 4 hours at +37°C in a total volume of 100 µl 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithioerythritol, pH approximately 7.5.

Exonucleases are not detectable using up to 60 U Reverse Transcriptase, M-MuLV.

Performance in RT-PCR

Reverse Transcriptase, M-MuLV is function tested by amplification of a 9.56 kb dystrophin gene fragment by RT-PCR with total RNA from human skeletal muscle.

References

1 Houts, G. E. *et al.* (1979) *J. Virol.* **29**, 517–522.
2 Roth, M. J. *et al.* (1985) *J. Biol. Chem.* **260**, 9326–9335.
3 Maniatis, T., Fritsch, E. F., & Sambrook, J. (1989) Molecular Cloning, A Laboratory Manual (Cold Spring Harbor Laboratory) p 8.11–8.13.
4 Gubler, U. & Hoffmann, B. J. (1983) *Gene* **25**, 263–269.

Changes to Previous Version

- Update of Quality Control procedure
- Update of Ordering Information
- Editorial Changes

Related Products

Product	Pack Size	Cat. No.
cDNA Synthesis Kit	1 kit	11 117 831 001
Primer for cDNA Synthesis $p(dT)_{15}$	20 µg	10 814 270 001
Protector RNase Inhibitor	2,000 U	03 335 399 001
	10,000 U	03 335 402 001
Deoxynucleoside Triphosphate Set	4 × 25 µmol	11 969 064 001
	4 × 125 µmol	03 622 614 001

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