MMuLV Reverse Transcriptase, 200 U/µl

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0400201	10000 U (50 rxn)	50 µl MMuLV Reverse Transcriptase 100 µl 10× MMuLV RT Buffer
BR0400202	50000 U (250 rxn)	5 × 50 µl MMuLV Reverse Transcriptase 5 × 100 µl 10× MMuLV RT Buffer

COMPONENT	COMPOSITION
MMuLV Reverse Transcriptase	MMuLV Reverse Transcriptase, 200 U/µl, in storage buffer containing 50% (v/v) glycerol.
10× MMuLV RT Buffer	Optimized 10× MMuLV Reverse Transcriptase reaction buffer.
STORAGE	-20°C (until expiry date – see product label)

FEATURES

- Pure reverse transcriptase for cDNA synthesis
- High yields of first-strand cDNA
- High value for a fair price

APPLICATIONS

- First-strand cDNA synthesis
- Generation of labeled cDNA
- RNA analysis by primer extension

DESCRIPTION

biotechrabbit™ MMuLV Reverse Transcriptase is an exceptionally pure DNA polymerase which uses RNA as a substrate and exhibits no measurable proofreading 3' →5' exonuclease function. This enzyme performs cDNA synthesis by extending a DNA primer annealed to an RNA template; it can also make copies from a single-stranded DNA templates.
The enzyme is purified from a recombinant *E. coli* strain carrying the MMuLV reverse transcriptase gene.

PROTOCOL

Prevention of cDNA synthesis reaction contamination

RNase contamination is an exceptional concern when working with RNA. RNase A, providing most threat to RNA integrity, is a highly stable contaminant of any laboratory. To prevent RNA from degradation and to minimize possibility of contamination during cDNA synthesis; follow the guidelines below:

- Use separate clean areas for preparation of the samples and the reaction mixture.
- DEPC-treat all tubes and pipette tips or use certified nuclease-free labware with aerosol filters.
- Wear fresh gloves when handling RNA and all reagents.
- Always assess the integrity of RNA prior to cDNA synthesis in denaturing agarose gel electrophoresis.
- Use RNase free water and other reagents.
- To prevent RNA from degradation, add Ribonuclease inhibitor (optional) in to the cDNA synthesis reaction (20 units for 20 µl reaction).

Typical cDNA synthesis reaction set up

- Thaw on ice and mix very well all reagents.
- Assemble and keep all reactions on ice.
- To use time and reagents effectively, always prepare master mix for multiple reactions. For a master mix volume, always calculate the number reactions that you need plus one additional.
- Combine the following in an RNase-free reaction tube:

COMPONENT	VOLUME	FINAL CONCENTRATION
dNTP Mix (10 mM each dNTP)	4 µl	2 mM (each dNTP)
RNase Inhibitor, 40 U/µl (optional)	1 µl	2 U/µl
Oligo (dT)$_{12-18}$ (10 µM) – or	*0.5 µl*	*0.25 µM*
Hexamer Primer (25 µM) – or	*1 µl*	*1.25 µM*
Gene Specific Primer (10 µM)	*0.5 µl*	*0.25 µM*
10× MMuLV RT Buffer	2 µl	1×
RNA Template	0.5–5 µg total RNA or 50–500 ng mRNA (polyA)	
MMuLV Reverse Transcriptase	1 µl	10 U/µl
RNase-free water	Variable	
Total volume	20 µl	

- Mix and collect the drops by centrifuging briefly.
- When using
  - Hexamer Primer, incubate 10 minutes at 25°C followed by 42°C for 45–60 minutes
  - Oligo (dT) or gene-specific Primer incubate at 42°C for 45–60 minutes.
- Inactivate enzyme at 85°C for 10 minutes.
- Collect the drops by spinning briefly.
- Store products at –20°C or proceed to next step, like PCR or qPCR.
- *Use maximum 10 µl of the cDNA synthesis reaction mix for PCR in 50 µl volume.*

CERTIFICATE OF ANALYSIS

Unit Definition
One unit is the amount of enzyme activity that incorporates 1 nmole of dTTP into acid insoluble fraction in 10 minutes at 42°C when poly(A)+ RNA and oligo (dT)20 are used as template-primer.

Quality Control

Functional Assay
cDNA synthesis with specific primers, followed by quantitative PCR.

Exonuclease assay
Linearized lambda/HindII fragments are incubated with Reverse Transcriptase in a 50 µl reaction mixture for 4 h at 37°C. No degradation of DNA was observed.

Endonuclease assay
lambda DNA is incubated with Reverse Transcriptase in a 50 µl reaction mixture for 4 h at 37°C. No degradation of DNA was observed.

Nick Activity
Supercoiled plasmid DNA is incubated with Reverse Transcriptase in a 50 µl reaction mixture for 4 h at 37°C. No conversion of covalently closed circular DNA to nicked DNA was detected.

E. coli genomic DNA contamination assay
A sample of Reverse Transcriptase is analyzed with specific primers targeting the 16S rRNA gene in qPCR for the presence of contaminating *E. coli* DNA. No *E. coli DNA* was detectable.

RNase Assay
A sample of the enzyme was incubated with a RNA template. RNase activity was not observed after agarose gel electrophoresis.

Quality confirmed by: Head of Quality Control

SAFETY INSTRUCTIONS
For safety instructions please see Safety Data Sheets (SDS)/Sicherheitshinweise finden Sie in den SDS unter: http://www.biotechrabbit.com/support/documentation.html.

USEFUL HINTS
- Visit Applications at www.biotechrabbit.com for more products and product selection guides.
- Most biotechrabbit products are available in custom formulations and bulk amounts.

CONTACT BIOTECHRABBIT
biotechrabbit GmbH
Volmerstr. 9a
12489 Berlin, Germany

info@biotechrabbit.com
support@biotechrabbit.com
www.biotechrabbit.com

Phone: +49 30 555 7821-10
Fax: +49 30 555 7821-99

Certified Quality-System mdc ISO 13485 ISO 9001

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