RevertUP™ II Reverse Transcriptase, 200 U/µl

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0400501	10000 U	50 µl RevertUP II Reverse Transcriptase 1 ml 5× Reverse Transcriptase Buffer
BR0400502	50000 U	2 × 125 µl RevertUP II Reverse Transcriptase 2 × 1 ml 5× Reverse Transcriptase Buffer

COMPONENT	COMPOSITION
RevertUP II Reverse Transcriptase	RevertUP II Reverse Transcriptase, 200 U/µl, in storage buffer containing 50% (v/v) glycerol.
5× Reverse Transcriptase Buffer	Optimized 5× Reverse Transcriptase reaction buffer.
STORAGE	-20°C (until expiry date – see product label)

FEATURES

- Improved performance for synthesis of long cDNAs (≥ 19 kb)
- Excellent stability at high temperatures up to 60°C
- High sensitivity for cDNA synthesis from few copies of template

APPLICATIONS

- First-strand cDNA synthesis
- Generation of labeled cDNA
- RNA analysis by primer extension
- cDNA library construction
- RT-PCR

DESCRIPTION

biotechrabbit™ RevertUP II Reverse Transcriptase is a proprietary MMuLV reverse transcriptase engineered by point mutations resulting in increased temperature stability up to 60°C. This guarantees top performance with templates showing a high degree of secondary structures. The enzyme has no RNase H activity and ensures efficient synthesis of ≥19 kb cDNA.

RevertUP Reverse Transcriptase is a 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 of single-stranded DNA templates.

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)	2 µl	1 mM (each dNTP)
RNase Inhibitor, 40 U/µl (optional)	0.5 µl	1 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*
5× Reverse Transcriptase Buffer	4 µl	1×
RNA Template	0.1–1 µg total RNA or 50–500 ng mRNA (polyA)	
RevertUP II 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 30°C followed by 42–55°C for 20–60 minutes
  - Oligo (dT) or gene-specific Primer incubate at 42–55°C for 20–60 minutes.
- Inactivate enzyme at 99°C for 5 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

Exonuclease assay

Linearized lambda/HindII fragments are incubated with the 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 the 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 the 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.

Functional Assay

cDNA synthesis with specific primers, followed by quantitative PCR.

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.

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Certified Quality-System mdc ISO 13485 ISO 9001

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