cDNA Synthesis Kit

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0400401	10 rxn of 20 µl	10 µl RevertUP™ II Reverse Transcriptase 100 µl 5× Reverse Transcriptase Buffer 25 µl dNTP Mix (10 mM each) 5 µl RNase Inhibitor 10 µl Hexamer Primer 5 µl Oligo (dT) Primer 1.5 ml PCR Grade Water
BR0400403	125 rxn of 20 µl	125 µl RevertUP™ II Reverse Transcriptase 1 ml 5× Reverse Transcriptase Buffer 250 µl dNTP Mix (10 mM each) 62.5 µl RNase Inhibitor 125 µl Hexamer Primer 62.5 µl Oligo (dT) Primer 5 × 1.5 ml PCR Grade Water

COMPONENT	COMPOSITION
RevertUP II Reverse Transcriptase	RevertUP II Reverse Transcriptase, 200 U/µl in Storage buffer, containing 50% glycerol
5× Reverse Transcriptase Buffer	Optimized 5× Reverse Transcriptase buffer for cDNA synthesis
dNTP Mix (10 mM each)	Aqueous solution (pH 7.0) containing 10 mM each: dATP, dCTP, dGTP, dTTP sodium salts
RNase Inhibitor	RNase Inhibitor, 40 U/µl, in Storage buffer, containing 50% glycerol
Hexamer Primer	25 µM Random Hexamer Primer
Oligo (dT) Primer	10 µM Oligo (dT) Primer
PCR Grade Water	Ultrapure, sterile filtrated water, DNase-, RNase- and protease-free
STORAGE	-20°C (until expiry date – see product label)

FEATURES

- Highly efficient synthesis of long cDNAs (≥ 19 kb)
- Excellent yields at temperatures up to 55°C
- High sensitivity reverse transcription from low abundance template
- Superior performance in demanding applications, including templates with a high degree of secondary structure

APPLICATIONS

- First-strand cDNA synthesis for RT-PCR and qPCR
- Gene expression profiling
- RNA labeling and primer extension
- cDNA library construction

DESCRIPTION

biotechrabbit™ cDNA Synthesis Kit provides superior components that ensure efficient first-strand cDNA synthesis from mRNA or total RNA templates. biotechrabbit RevertUP™ II Reverse Transcriptase enables highly efficient reverse transcription with increased thermostability. biotechrabbit RNase Inhibitor is a potent non-competitive inhibitor of RNases. The combination of highly efficient cDNA synthesis, effective RNase inhibition and pure dNTPs allows high yields of cDNAs of more than 19 kb.
For greater application flexibility, hexamer primers, allowing all RNAs in the reaction to be used as templates, and an oligo (dT) primer, for the synthesis of cDNA from only poly(A) tailed mRNA, are included.

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)	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
PCR Grade 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 50–55°C for 20–60 minutes
  - Oligo (dT) or gene-specific Primer incubate at 50–55°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

Quality Control

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.

CONTACT BIOTECHRABBIT

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 valid from 25.04.2019