AllScript™ Reverse Transcriptase, 4 U/µl

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0400601	400 U (100 rxn)	100 µl AllScript Reverse Transcriptase 1 ml 5× Reverse Transcriptase Buffer

COMPONENT	COMPOSITION
AllScript Reverse Transcriptase	AllScript Reverse Transcriptase, 4 U/µl, in storage buffer containing 50% (v/v) glycerol.
5× Reverse Transcriptase Buffer	Optimized 5× Reaction Buffer for AllScript Reverse Transcriptase.
STORAGE	-20°C (until expiry date – see product label)

FEATURES

- Highly specific and sensitive RT-PCR
- Excellent performance in transcription of complex RNA secondary structures
- High yields of cDNA even with targets in low copy number
- RNase H activity specific to RNA hybridized to cDNA for improved 1step PCR

APPLICATIONS

- Standard reverse transcription
- Synthesis of ds cDNA for cloning
- RT-PCR and qRT-PCR
- Rapid amplification of cDNA ends (RACE)
- RNA analysis by primer extension

DESCRIPTION

biotechrabbit™ AllScript Reverse Transcriptase is a proprietary RT designed for highly specific and sensitive reverse transcription. It guarantees top performance in standard reverse transcription, synthesis of ds cDNA for cloning, RT-PCR and qRT-PCR, rapid amplification of cDNA ends (RACE) or RNA analysis by primer extension. It's high affinity to RNA allows transcription of complex RNA secondary structures and targets in low copy number, leading to high yields of cDNA.

AllScript Reverse Transcriptase is a multifunctional enzyme including RNA-dependent and ssDNA-dependent DNA polymerase, as well as RNase H activity. The RNase H activity is specific to RNA hybridized to cDNA, with no effect on pure RNA template, resulting in improved performance of subsequent PCR.

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)	1 µl	0.5 mM (each dNTP)
RNase Inhibitor, 40 U/µl (optional)	0.5 µl	1 U/µl
Oligo (dT)$_{12-18}$ (10 µM) – or *Hexamer Primer (100 µM) – or* *Gene Specific Primer (10 µM)*	*2 µl* *2 µl* *0.2 - 2 µl*	*1 µM* *10 µM* *0.1 – 1 µM*
5× Reverse Transcriptase Buffer	4 µl	1×
RNA Template	50 ng – 2 µg total RNA or 50–500 ng mRNA (polyA)	
AllScript Reverse Transcriptase	0.5 – 1 µl	0.125 – 0.25 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 37–50°C for 30 minutes (increase up to 60 min, if needed)
  - Oligo (dT) or gene-specific Primer incubate at 37–50°C for 30 minutes (increase up to 60 min, if needed)
- Reaction temperature can be raised to 55°C (small activity reduction may occur)
- In case of higher secondary structures, perform a pre-incubation of the RNA template for 5 min at 65°C (place on ice immediately after) and/or increase the incubation time for the subsequent RT reaction (at 37–50°C)
- Inactivate enzyme at 95°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 nmol 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 Activity

Linearized lambda/HindIII DNA fragments are incubated with the enzyme in a 50 µl reaction mixture for 4 h at 37°C. No DNA degradation observed.

Endonuclease/Nick Activity

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

Contamination with E. coli DNA

Absence of *E. coli* genomic DNA is confirmed by qPCR using a sample of the enzyme and specific primers targeting the *E. coli* 16S rRNA gene. No contamination detected.

RNase Assay

An RNA template is incubated with the enzyme in a 20 µl reaction mixture for 1 h at 42°C. No RNA degradation observed.

Functional Assay

cDNA synthesis with Oligo (dT) and/or Hexamer primers, followed by 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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