[Cat. No.] E-3161, E-3162

Introduction

RocketScript™ Reverse Transcriptase, RNase H Minus is an RNA and DNA-dependent polymerase removed with RNase H activity by introducing mutation in RNase H domain. This engineered enzyme provides enhanced extensibility, resulting in higher yields of cDNA and longer product (up to 13 kb) than *RocketScript*™ Reverse Transcriptase. It can be used to synthesize cDNA at a temperature range of 42-70°C, providing increased specificity and it can successfully synthesize cDNA even with 1 pg of human total RNA due to its sensitivity.

Applications

- Gene synthesis
- First-strand cDNA synthesis from RNA molecules
- RT-PCR
- Random priming reactions
- Library construction
- Probe labeling
- mRNA 5'end mapping by primer extension analysis
- Real-time PCR

Components

Components	E-3161	E-3162
RocketScript™ Reverse Transcriptase, RNase H Minus	10,000 U (50 µl)	50,000 U (50 µl x 5)
5X Reaction buffer	0.5 ml	0.5 ml x 5
10 mM dNTPs	0.2 ml	0.2 ml x 5
100 mM DTT	0.2 ml	0.2 ml x 5
RNase inhibitor	50 µl	50 µl x 5

*** Note:** For research use only. Not for use in diagnostic or therapeutic procedures.

Specifications

RocketScript™ Reverse Transcriptase, RNase H Minus	
5' to 3' exonuclease activity	No
3' to 5' exonuclease activity	No
3'–A overhang	No
Fragment size	Up to 12.5 kb

Buffer Composition

5X Reaction buffer	250 mM Tris-HCl, 375 mM KCl, 15 mM $MgCl_2$, and stabilizer pH 8.3

Storage Buffer

RocketScript™ Reverse Transcriptase, RNase H Minus is supplied in 50% (v/v) glycerol containing 20 mM Tris-HCl, 150 mM NaCl, 0.1 mM EDTA, 1 mM DTT, and stabilizer, pH 7.6.

Unit Definition

One unit is defined as the amount of enzyme that incorporates 1 nmole of dTTP into acid-insoluble products in 10 min at 37°C using poly(A)·oligo(dT) as template primer.

Quality Control

- Nuclease Contamination Assay: Nuclease activity is not detected after incubation of 1 µg of DNA and RNA with 200 U of *RockerScript*™ Reverse Transcriptase, RNase H minus at 37-42°C for 3 hrs.

Storage

Store at -20°C. If stored in the recommended temperature, this product will be stable until the expiration date printed out on the label.

Online Resources

Korean **English**

Visit our **product page** for additional information and protocols.

Ordering Information

Description		Cat. No
RocketScript™ Reverse Transcriptase, RNase H Minus	10,000 U (50 rxn)	E-3161
	50,000 U (250 rxn)	E-3162

Notice

BIONEER corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

Explanation of Symbols

Experimental Procedures

Step 1: Preparation of reaction mixture

1. Mix template RNA and primers in a sterile tube (not provided) indicated as below.

- Amount of template RNA and primers

Components		20 µl reaction
Template RNA	Total RNA	1-5 µg
	mRNA	5-100 ng
Primers	Oligo dT or Random primer	10-100 pmol
	Sequence specific	10-30 pmol

2. Add all components for cDNA synthesis into PCR tubes (not provided) to a total volume of 20 µl.

- Preparation of reaction mixture

Components	20 µl reaction
Mixture of template RNA and primers	Variable
5X Reaction buffer	4 µl
10 mM dNTPs	2 µl or Variable
100 mM DTT	2 µl
RNase inhibitor (100 ng/µl)	0.5-1 µl
RocketScript™ Reverse Transcriptase, RNase H Minus (200 U/µl)	200 U
Nuclease-free water	Variable
Total volume	20 µl

Step 2: cDNA synthesis

3. Perform the reaction under the following conditions.

3-1. Cyclic reverse transcription (example 1)

Step	Temperature				Time	Cycles
	dN_6	dN_{12}	dT_{20}	Sequence specific		
Primer annealing	15°C	30°C	37°C	Tm of primers	10-30 sec	12 cycles or less
cDNA synthesis			50°C		4 min	
Melting secondary structure & cDNA synthesis			55°C		30 sec	
Heat inactivation			95°C		5 min	1 cycle

3-2. Single temperature reaction (example 2)

Step	Temperature				Time	Cycles
	dN_6	dN_{12}	dT_{20}	Sequence specific		
Primer annealing	15°C	30°C	37°C	Tm of primers	1 min	1 cycle
cDNA synthesis			42-70°C		10-60 min	1 cycle
Heat inactivation			95°C		5 min	1 cycle

4. After the reaction, maintain the reaction mixture at 4°C. The samples can be stored at -20°C until use.