CycleScript™ Reverse Transcriptase

[Cat. No.] E-3131, E-3132

Introduction

CycleScript™ Reverse Transcriptase is applied with BIONEER's patent technology called Cyclic Reverse Transcription (CRT), which not only increases the efficiency, but also is effective for full-length cDNA synthesis. The CRT reaction can perform homogeneous primer-annealing at low temperature as well as reverse transcription with high sensitivity at high temperature, which even complex RNA secondary structure is resolved. It is composed of 2 or 3 steps. The step 1 is performed at 15-25°C, at which short primers are fully annealed. Then, the step 2 is conducted at 42-48°C for cDNA synthesis and the step 3 is followed at high temperature 50-55°C at which secondary structure of template RNA obstructing reverse transcription is released.

Applications

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

Components

Components	E-3131	E-3132
CycleScript™ Reverse Transcriptase	10,000 U (50 µl)	50,000 U (50 µl x 5)
5X Reaction buffer	0.4 ml	0.4 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

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

Specifications

CycleScript™ Reverse Transcriptase	
5' to 3' exonuclease activity	No
3' to 5' exonuclease activity	No
3'–A overhang	No
Strand displacement	Yes
Fragment size	Up to 9 kb

Buffer Composition

5X Reaction buffer	150 mM Tris, 250 mM KCl, 10 mM $MgCl_2$, etc, pH 8.1

Storage Buffer

CycleScript™ Reverse Transcriptase is supplied in 50% (v/v) glycerol containing 20 mM Tris-HCl, 150 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 50 mM $(NH_4)_2SO_4$, 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 *CycleScript*™ Reverse Transcriptase 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
CycleScript™ Reverse Transcriptase	10,000 U (50 rxn)	E-3131
	50,000 U (250 rxn)	E-3132

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

LOT Batch Code | REF Catalog Number | Caution | Consult Instructions For Use

Contains Sufficient for <n> tests | RUO Research Use Only | Temperature Limitation | Use-by Date

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 µg
	RNA	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
Step 1 mixture	Variable
5X Reaction buffer	4 µl
10 mM dNTPs	2 µl or Variable
100 mM DTT	2 µl
RNase inhibitor (Not provided)	Variable
CycleScript™ Reverse Transcriptase (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
Primer annealing	15-25°C	30 sec	12 cycles or less
cDNA synthesis	42-45°C	4 min	
Melting secondary structure & cDNA synthesis	55°C	30 sec	
Heat inactivation	95°C	5 min	1 cycle

3-2. Cyclic reverse transcription (example 2)

Step	Temperature	Time	Cycles
Primer annealing	15-25°C	1 min	12 cycles or less
Melting secondary structure & cDNA synthesis	42-50°C	4 min	
Heat inactivation	95°C	5 min	1 cycle

* **Note:** For difficult or high GC-content templates, perform cDNA synthesis at 55°C.

3-3. Single temperature reaction (example 3)

Step	Temperature	Time	Cycles
cDNA synthesis	37-50°C	30-60 min	1 cycle
Heat inactivation	95°C	5 min	1 cycle

* **Note:** Recommended temperature is range of 42-48°C.

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