AccuPower® *CycleScript*™ RT PreMix (dN_{12}) (V4/2022-03-02)

[Cat. No.] Please refer to the **Ordering Information**

Introduction

AccuPower® *CycleScript*™ RT PreMix (dN_{12}) is applied with BIONEER's patent technology called Cyclic Temperature Reverse Transcription (CTRT), which not only increases the efficiency, but also is effective for full-length cDNA synthesis. The CTRT reaction 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 performed at 42-48°C for cDNA synthesis. The step 3 is performed at high temperature 50-55°C at which secondary structure of template RNA obstructing reverse transcription is released. This product contains vacuum-dried components including thermostable *CycleScript*™ Reverse Transcriptase, reaction buffer, DTT, dNTPs, Oligo dN_{12} primers, and stabilizer. It simplifies preparation of reverse transcription reaction mixture by adding template RNA and nuclease-free water without any extra process.

Applications

- Sequencing single and double-strand DNA or RNA
- Random priming reaction
- cDNA library construction
- Probe labeling
- mRNA 5'–end mapping by primer extension analysis
- PCR
- Real-time PCR

Features & Benefits

- Flexible conditions: Cyclic Temperature Reverse Transcription (CTRT) is more efficient cDNA synthesis by using higher temperature than the conventional methods using at 42°C. Primer annealing is performed at a range of low temperature (15-40°C) and secondary structures of template is released by repeating the step 2 or 3 at a range of high temperature (50-55°C).
- Stability: Included stabilizer and thermostable reverse transcriptase allow to react at a higher temperature up to 55°C.
- Controllable reaction time: Reaction time can be controlled depending on the number and size of copies of target gene. In case of high-copy gene, cDNA can be synthesized even with 10 minutes reverse transcription reaction.
- Reproducibility: Mass production under ISO 9001 quality system allows minimized deviation between lots and reproducible results in replicated tests performed under same conditions and variation.
- Ease-of-use: Reactants are individually packaged in each of the PCR tubes, it allows any user simply perform cDNA synthesis by adding template RNA and nuclease-free water.

Composition

Composition	Concentration
CycleScript™ Reverse Transcriptase	200 U
5X Reaction buffer	1X
DTT	0.25 mM
dNTPs (dATP, dCTP, dGTP, dTTP)	Each 250 µM
RNase inhibitors	1 U
Oligo dN_{12}	100 pmol

Specifications

CycleScript™ Reverse Transcriptase	
DNase activity	No
RNase activity	No
Fragment size	Up to 9 kb

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

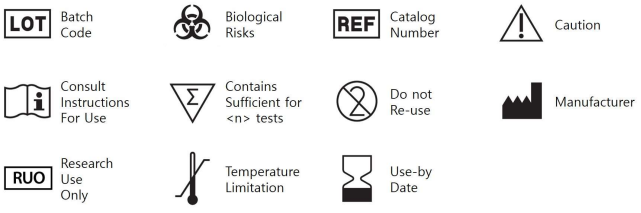
Ordering Information

Description			Cat. No.
0.2 ml thin-wall 8-tube strips with attached cap	96 tubes	20 µl/rxn	K-2045
		50 µl/rxn	K-2048
	480 tubes	20 µl/rxn	K-2045-B
		50 µl/rxn	K-2048-B
0.5 ml thin-wall tubes with attached cap	100 tubes	50 µl/rxn	K-2050-1

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



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Experimental Procedures

Step 1 — Preparation of reaction mixture

1. Add template RNA and nuclease-free water into *AccuPower® CycleScript™* RT PreMix (dN_{12}) tubes to make a total volume of 20 µl or 50 µl. Do not include the dried pellet.

- Amount of template RNA

Components		20 µl reaction	50 µl reaction
Template RNA	Total RNA	0.1-1.0 µg	0.1-1.0 µg
	Poly(A) RNA	0.01-1.0 µg	0.01-1.0 µg

2. Dissolve the vacuum-dried pellet by pipetting or vortexing, and briefly spin down.

Step 2 — cDNA synthesis

3. Perform the reaction under the following conditions.

3-1. CTRT reaction (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. CTRT reaction (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

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.

Option

- If PCR is needed, transfer 2-5 µl of reaction mixture (synthesized cDNA) into *AccuPower®* PCR PreMix tubes (Cat. No. K-2012, not provided), and perform the reaction under the following conditions.

Step	Temperature	Time	Cycles
Pre-denaturation	95°C	5 min	1 cycle
Denaturation	95°C	20 sec	25-35 cycles
Annealing	45-65°C	20 sec	
Extension	72°C	0.5-1 min/kb	
Final extension	72°C	3-5 min	1 cycle

*** Note:** For maximum yield and specificity, temperatures and cycling times should be optimized for each new template DNA or primers.

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