AccuPower® *CycleScript*™ RT Master Mix

[Cat. No.] K-2051

Introduction

AccuPower® *CycleScript*™ RT Master Mix 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 is a ready-to-use mixture containing thermostable *CycleScript*™ Reverse Transcriptase, reaction buffer, DTT, dNTPs, and stabilizer. Primers (Oligo dT_{20} and Oligo dN_6) are provided in separated tubes. It simplifies preparation of reverse transcription reaction mixture by adding template RNA, primers, 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 included in a tube, it allows any user simply perform cDNA synthesis by adding template RNA and nuclease-free water.

Components

Components	Amount
2X Master Mix	1 ml
Oligo dT_{20} (100 pmol/μl)	100 μl
Oligo dN_6 (100 pmol/μl)	100 μl

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

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

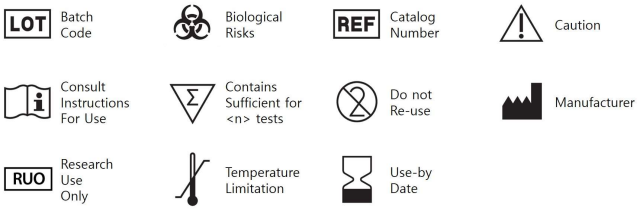
Description		Cat. No.
1 ml of 2X Master Mix solution	1 ml x 1 ea	K-2051

*** Note:** For Master Mix products, primers (dT_{20} & dN_6) are provided in a separate tube.

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 — Thaw reagents

1. Thaw *AccuPower® CycleScript™* RT Master Mix on ice and mix thoroughly before use. Then, briefly spin down components.
2. Dispense appropriate volumes of *AccuPower® CycleScript™* RT Master Mix into PCR tubes (not provided). Use 10 µl and 25 µl of 2X Master Mix for 20 µl reaction and 50 µl reaction, respectively.

Step 2 — Preparation of reaction mixture

3. Add template RNA, primers, and nuclease-free water into PCR tubes containing *AccuPower® CycleScript™* RT Master Mix.

- Amount of template RNA and primers

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
Primers	Oligo dT	50-100 pmol	250 pmol
	Random primer	100 pmol	250 pmol
	Gene specific primer	10-50 pmol	10-50 pmol

4. Mix the reaction mixture by pipetting or vortexing, and briefly spin down.

Step 3 — cDNA synthesis

5. Perform the reaction under the following conditions.

5-1. 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

5-2. 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

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

5-3. 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.

6. 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.