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## I. Introduction

AccuPower® CycleScript™ RT Master Mix is a ready-to-use reverse transcription kit, which can generate homogeneous cDNA synthesis through temperature cycling (patent pending). This product contains all components including thermostable CycleScript™ Reverse Transcriptase, dNTPs, reaction buffer, stabilizers for reverse transcription AccuPower® CycleScript™ RT Master Mix has high reverse transcription activity in broad ranges of temperature between conventional 42°C and 55°C. This product is designed for Cyclic Temperature Reverse Transcription (CTRT), which the CTRT reaction can be performed in higher performance than that of reverse transcription reaction at conventional single temperature. The CTRT reaction is composed of 2 or 3 steps as follows; The Step 1 is performed at 15~25°C, at which short primer is fully annealed. And then, the Step 2 is performed at 42~48°C (optional) for cDNA synthesis. The Step 3 is performed at high temperature 50~55°C at which secondary structure of RNA template obstructing reverse transcription is melted and reverse transcription is also occurred.

## II. Application

- Standard RT and RT-PCR
- Real-Time PCR
- Synthesis of double-stranded cDNA for cloning
- Gene expression level analysis

## III. Contents

Components		Amount
Master Mix	CycleScript™ Reverse Transcriptase	200 U
	5 x Reaction Buffer	1 x
	DTT	0.25 mM
	dNTP	250 µM each
	RNase Inhibitor	1 U
Primer (Oligo dT20)		100 pmole
Primer (Oligo dN6)		100 pmole

## V. Storage

AccuPower® CycleScript™ RT Master Mix, should be stored at -20°C upon receipt and is stable until the expiration date stated on the label.

## VI. Additional Required Materials & Devices

- Thermal cycler for PCR
- Target-specific primer
- Calibrated micropipette
- Sterilized micropipette tips with filters
- DEPC-water

## VII. General Precautions

- Wear gloves during experiments to prevent contamination.
- Store positive materials, such as samples and control templates, in separated freezer from freezers for the kit.
- Add templates to the reaction mixture in clean bench or a spatially separated facility.

## X. Ordering Information

Cat. No.	Description
K-2051	AccuPower® CycleScript™ RT Master Mix (1 ml, 2X)

## VIII. Protocol

1. Thaw total RNA, DEPC-water, and primer before use.
2. Add total RNA and primer (oligo dT, random primer, or specific primer) into place in the PCR tube containing the mastermix.

### 1) Recommended amount of DEPC-D.W. (dT20, dN6)

Component	Amount	
	dT20	dN6
Primer	100 pmole/µl	
DEPC-D.W.	100 µl	100 µl
Total	100 µl	100 µl

\* vortex and spin down

### 2) Recommended amount of template and primer

Component	Amount	
	10 µl	25 µl
2X Master Mix <sup>(1)</sup>	10 µl	25 µl
Primer <sup>(2)</sup>	Oligo dT	50-100 pmole
	Random	100 pmole
	Specific	10 - 50 pmole
Template RNA <sup>(3)</sup>	10 pg - 5 µg	25 pg - 12.5 µg
DEPC-D.W.	20-((1)+(2)+(3)) µl	50-((1)+(2)+(3)) µl
Total	20 µl	50 µl

3. Spin down by using Bioneer's ExiSpin™ Vortex/Centrifuge or by pipetting up and down several times and briefly spinning down.

### 1) CTRT reactions

Step	Temperature			Time	No. of Cycles
	dN <sub>6</sub>	dN <sub>12</sub>	dT <sub>20</sub>		
Primer annealing	15°C	20°C	25°C	T <sub>m</sub> of primer	30 sec
cDNA synthesis	42~45°C			4 min	Repeat 12 times or less
Melting	55°C			30 sec	
secondary structure & cDNA synthesis	95°C			5 min	
Heat inactivation	4°C				
Store					

### 2) Alternative protocol

Step	Temperature	Time	No. of Cycles
Primer annealing	15 ~ 25 °C	1 min	Repeat 12 times or less
cDNA synthesis	42 ~ 50°C	4 min	
Heat inactivation	95°C	5 min	
Incubate	4°C		

Note: for difficult or high GC-content templates, use a 55°C cDNA synthesis temperature.

### 3) Single temperature reaction

37~50°C (You can choose one temperature but this product prefers 42~48°C reaction) 30~60 min. → 95°C 5 min

## IX. Notice

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