

[Cat. No.] **K-2216**

Introduction

AccuPower® RocketScript™ Cycle RT Master Mix is a next generation cDNA synthesis kit that uses RocketScript™ Reverse Transcriptase (RocketScript™ RTase) and Cyclic Temperature Reverse Transcription (CTRT) technologies developed by BIONEER. RocketScript™ RTase originated and engineered from M-MLV reverse transcriptase can provide increased thermal stability resulting in effective full length first strand cDNA synthesis. It can be used at various temperatures (42-70°C) allowing increased specificity, higher yields of cDNA, and more full-length product than other reverse transcriptase. In addition, the CTRT not only can increase the efficiency, but also 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 RocketScript™ RTase, reaction buffer, DTT, dNTPs, and RNase inhibitors. Primers (Oligo dT₂₀ and Oligo dN₆) are provided in separate tubes. It simplifies preparation of reverse transcription reaction mixture by adding template RNA, primers, and nuclease-free water without any extra process.

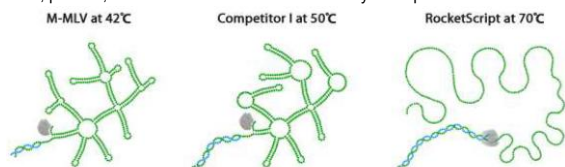


Figure 1. Schematic diagram of 5' UTR of a gene, with complex secondary structure, at three different temperatures.

RocketScript™ Reverse Transcriptase is fully active at 70°C, so it can synthesize complete gene sequences that M-MLV and other reverse transcriptase can not synthesize.

Applications

- First-strand synthesis of cDNA from RNA molecules (RT)
- Random priming reaction
- cDNA library construction
- Probe labeling
- mRNA 5'-end mapping by primer extension analysis
- PCR
- Real-time PCR
- Gene sequencing

Features and Benefits

- **Sensitivity & Efficiency:** RocketScript™ RTase and CTRT technologies provide enhanced sensitivity synthesizing cDNA from low concentration RNA and increased efficiency by applying circulating temperature reaction.
- **Thermostable activity:** RocketScript™ RTase has excellent thermal resistance even at 70°C, so it is suitable for cDNA synthesis of secondary RNA structures. The cDNA synthesis temperatures can be set at various temperatures (42-70°C) according to user's experimental conditions, allowing effective cDNA synthesis.
- **Flexible Reaction:** As well as CTRT, reverse transcription reaction is possible at a single temperature within 22-55°C.

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

Components	Amount
RocketScript™ 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

RocketScript™ Reverse Transcriptase	
DNase activity	No
RNase activity	No
Fragment size	Up to 10 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-2216

* **Note:** For Master Mix products, primers (dT₂₀ & dN₆) 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



Batch Code



Biological Risks



Catalog Number



Caution



Consult Instructions For Use



Contains Sufficient for <n> tests



Do not Re-use



Manufacturer



Research Use Only



Temperature Limitation



Use-by Date

Experimental Procedures

Steps		Procedure Details																																																			
1	 Thaw reagents	1. Thaw <i>AccuPower® RocketScript™</i> Cycle RT Master Mix on ice and mix thoroughly before use. Then, briefly spin down components. 2. Dispense appropriate volumes of <i>AccuPower® RocketScript™</i> Cycle 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.																																																			
2	 Preparation of Reaction mixture	3. Add template RNA, primers, and nuclease-free water into PCR tubes containing <i>AccuPower® RocketScript™</i> Cycle RT Master Mix. • Amount of template RNA and primers <table><tr><th colspan="2">Total</th><th>20 µl reaction</th><th>50 µl reaction</th></tr><tr><td rowspan="2">Template RNA</td><td>Total RNA</td><td>0.01-5 µg</td><td>0.01-5 µg</td></tr><tr><td>Poly(A) RNA</td><td>0.01-5 µg</td><td>0.01-5 µg</td></tr><tr><td rowspan="3">Primers</td><td>Oligo dT</td><td>50-100 pmol</td><td>250 pmol</td></tr><tr><td>Random primer</td><td>100 pmol</td><td>250 pmol</td></tr><tr><td>Gene specific primer</td><td>10-50 pmol</td><td>10-50 pmol</td></tr></table> 4. Mix the reaction mixtures by pipetting or vortexing, and briefly spin down				Total		20 µl reaction	50 µl reaction	Template RNA	Total RNA	0.01-5 µg	0.01-5 µg	Poly(A) RNA	0.01-5 µg	0.01-5 µg	Primers	Oligo dT	50-100 pmol	250 pmol	Random primer	100 pmol	250 pmol	Gene specific primer	10-50 pmol	10-50 pmol																											
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3	 cDNA synthesis	5. Perform the reaction under the following conditions. 5.1 CTRT reaction (Example 1) <table><tr><th>Step</th><th>Temperature</th><th>Time</th><th>Cycle</th></tr><tr><td>Primer annealing</td><td>37°C</td><td>10-30 sec</td><td></td></tr><tr><td>cDNA synthesis</td><td>50°C</td><td>4 min</td><td>10 cycles or more</td></tr><tr><td>Melting secondary structure & cDNA synthesis</td><td>55-60°C</td><td>30 sec</td><td></td></tr><tr><td>Heat inactivation</td><td>95°C</td><td>5 min</td><td>1 cycle</td></tr></table> 5.2 CTRT reaction (Example 2) <table><tr><th>Step</th><th>Temperature</th><th>Time</th><th>Cycle</th></tr><tr><td>Primer annealing</td><td>37°C</td><td>1 min</td><td></td></tr><tr><td>Melting secondary structure & cDNA synthesis</td><td>42-70°C</td><td>4 min</td><td>10 cycles or more</td></tr><tr><td>Heat inactivation</td><td>95°C</td><td>5 min</td><td>1 cycle</td></tr></table> *Note: Annealing temperature setting(5-1, 5-2), dN_{TP}(15 °C), dN_{TP}(30 °C), Gene specific (T_m of primers) 5.3 Single temperature reaction (Example 3) <table><tr><th>Step</th><th>Temperature</th><th>Time</th><th>Cycle</th></tr><tr><td>cDNA synthesis</td><td>22-55°C</td><td>30-60 min</td><td>1 cycle</td></tr><tr><td>Heat inactivation</td><td>95°C</td><td>5 min</td><td>1 cycle</td></tr></table> 6. After the reaction, maintain the reaction mixture at 4°C. The samples can be stored at -20°C until use.				Step	Temperature	Time	Cycle	Primer annealing	37°C	10-30 sec		cDNA synthesis	50°C	4 min	10 cycles or more	Melting secondary structure & cDNA synthesis	55-60°C	30 sec		Heat inactivation	95°C	5 min	1 cycle	Step	Temperature	Time	Cycle	Primer annealing	37°C	1 min		Melting secondary structure & cDNA synthesis	42-70°C	4 min	10 cycles or more	Heat inactivation	95°C	5 min	1 cycle	Step	Temperature	Time	Cycle	cDNA synthesis	22-55°C	30-60 min	1 cycle	Heat inactivation	95°C	5 min	1 cycle
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	 Option	• If PCR is needed, transfer 2-5 µl of reaction mixture (synthesized cDNA) into <i>AccuPower®</i> PCR PreMix tubes (Cat. No. K-2012, not provided), and perform the reaction under the following conditions. <table><tr><th>Step</th><th>Temperature</th><th>Time</th><th>Cycle</th></tr><tr><td>Pre-denaturation</td><td>95°C</td><td>5 min</td><td>1 cycle</td></tr><tr><td>Denaturation</td><td>95°C</td><td>20 sec</td><td></td></tr><tr><td>Annealing</td><td>45-65°C</td><td>20 sec</td><td>25-35 cycles</td></tr><tr><td>Extension</td><td>72°C</td><td>0.5-1 min/kb</td><td></td></tr><tr><td>Final Extension</td><td>72°C</td><td>3-5 min</td><td>1 cycle</td></tr></table> * Note: For maximum yield and specificity, temperatures and cycling times should be optimized for each new template DNA or primers.				Step	Temperature	Time	Cycle	Pre-denaturation	95°C	5 min	1 cycle	Denaturation	95°C	20 sec		Annealing	45-65°C	20 sec	25-35 cycles	Extension	72°C	0.5-1 min/kb		Final Extension	72°C	3-5 min	1 cycle																								
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