

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

Introduction

AccuPower® CycleScript™ RT PreMix (dT₂₀) 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 dT₂₀ 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 dT ₂₀	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

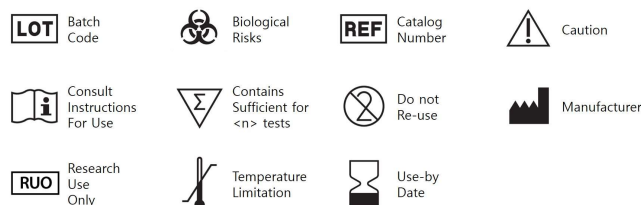
Ordering Information

Description	Cat. No.
0.2 ml thin-wall 96 tubes	K-2044
8-tube strips with attached cap	K-2047
480 tubes	K-2044-B
50 µl/rxn	K-2047-B
0.5 ml thin-wall tubes with attached cap	K-2050




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

Steps		Procedure Details																																																			
1	 Preparation of reaction mixture	<p>1. Add template RNA and nuclease-free water into <i>AccuPower® CycleScript™ RT PreMix (dT₂₀)</i> tubes to make a total volume of 20 µl or 50 µl. Do not include the dried pellet.</p> <ul style="list-style-type: none"> Amount of template RNA <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th colspan="2">Components</th> <th>20 µl reaction</th> <th colspan="2">50 µl reaction</th> </tr> </thead> <tbody> <tr> <td rowspan="2" style="text-align: center;">Template RNA</td> <td style="text-align: center;">Total RNA</td> <td style="text-align: center;">0.1-1.0 µg</td> <td colspan="2" style="text-align: center;">0.1-1.0 µg</td> </tr> <tr> <td style="text-align: center;">Poly(A) RNA</td> <td style="text-align: center;">0.01-1.0 µg</td> <td colspan="2" style="text-align: center;">0.01-1.0 µg</td> </tr> </tbody> </table> <p>2. Dissolve the vacuum-dried pellet by pipetting or vortexing, and briefly spin down.</p>				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																																			
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2	 cDNA synthesis	<p>3. Perform the reaction under the following conditions.</p> <p>3-1. CTRT reaction (Example 1)</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Step</th> <th>Temperature</th> <th>Time</th> <th>Cycles</th> </tr> </thead> <tbody> <tr> <td>Primer annealing</td> <td style="text-align: center;">15-25°C</td> <td style="text-align: center;">30 sec</td> <td></td> </tr> <tr> <td>cDNA synthesis</td> <td style="text-align: center;">42-45°C</td> <td style="text-align: center;">4 min</td> <td style="text-align: center;">12 cycles or less</td> </tr> <tr> <td>Melting secondary structure & cDNA synthesis</td> <td style="text-align: center;">55°C</td> <td style="text-align: center;">30 sec</td> <td></td> </tr> <tr> <td>Heat inactivation</td> <td style="text-align: center;">95°C</td> <td style="text-align: center;">5 min</td> <td style="text-align: center;">1 cycle</td> </tr> </tbody> </table> <p>3-2. CTRT reaction (Example 2)</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Step</th> <th>Temperature</th> <th>Time</th> <th>Cycles</th> </tr> </thead> <tbody> <tr> <td>Primer annealing</td> <td style="text-align: center;">15-25°C</td> <td style="text-align: center;">1 min</td> <td></td> </tr> <tr> <td>Melting secondary structure & cDNA synthesis</td> <td style="text-align: center;">42-50°C</td> <td style="text-align: center;">4 min</td> <td style="text-align: center;">12 cycles or less</td> </tr> <tr> <td>Heat inactivation</td> <td style="text-align: center;">95°C</td> <td style="text-align: center;">5 min</td> <td style="text-align: center;">1 cycle</td> </tr> </tbody> </table> <p>3-3. Single temperature reaction (Example 3)</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Step</th> <th>Temperature</th> <th>Time</th> <th>Cycles</th> </tr> </thead> <tbody> <tr> <td>cDNA synthesis</td> <td style="text-align: center;">37-50°C</td> <td style="text-align: center;">30-60 min</td> <td style="text-align: center;">1 cycle</td> </tr> <tr> <td>Heat inactivation</td> <td style="text-align: center;">95°C</td> <td style="text-align: center;">5 min</td> <td style="text-align: center;">1 cycle</td> </tr> </tbody> </table> <p>* Note: Recommended temperature is range of 42-48°C.</p> <p>4. After the reaction, maintain the reaction mixture at 4°C. The samples can be stored at -20°C until use.</p>				Step	Temperature	Time	Cycles	Primer annealing	15-25°C	30 sec		cDNA synthesis	42-45°C	4 min	12 cycles or less	Melting secondary structure & cDNA synthesis	55°C	30 sec		Heat inactivation	95°C	5 min	1 cycle	Step	Temperature	Time	Cycles	Primer annealing	15-25°C	1 min		Melting secondary structure & cDNA synthesis	42-50°C	4 min	12 cycles or less	Heat inactivation	95°C	5 min	1 cycle	Step	Temperature	Time	Cycles	cDNA synthesis	37-50°C	30-60 min	1 cycle	Heat inactivation	95°C	5 min	1 cycle
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 Option	<ul style="list-style-type: none"> If PCR is needed, transfer 2-5 µl of reaction mixture (synthesized cDNA) into <i>AccuPower® PCR PreMix</i> tubes (Cat. No. K-2012, not provided), and perform the reaction under the following conditions. <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Step</th> <th>Temperature</th> <th>Time</th> <th>Cycles</th> </tr> </thead> <tbody> <tr> <td>Pre-denaturation</td> <td style="text-align: center;">95°C</td> <td style="text-align: center;">5 min</td> <td style="text-align: center;">1 cycle</td> </tr> <tr> <td>Denaturation</td> <td style="text-align: center;">95°C</td> <td style="text-align: center;">20 sec</td> <td></td> </tr> <tr> <td>Annealing</td> <td style="text-align: center;">45-65°C</td> <td style="text-align: center;">20 sec</td> <td style="text-align: center;">25-35 cycles</td> </tr> <tr> <td>Extension</td> <td style="text-align: center;">72°C</td> <td style="text-align: center;">0.5-1 min/kb</td> <td></td> </tr> <tr> <td>Final extension</td> <td style="text-align: center;">72°C</td> <td style="text-align: center;">3-5 min</td> <td style="text-align: center;">1 cycle</td> </tr> </tbody> </table> <p>* Note: For maximum yield and specificity, temperatures and cycling times should be optimized for each new template DNA or primers.</p>				Step	Temperature	Time	Cycles	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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