AccuPower® RT Master Mix

[Cat. No.] K-2263

Introduction

AccuPower® RT Master Mix is a ready-to-use mixture for cDNA synthesis including *M-MLV* Reverse Transcriptase, reaction buffer, and RNase inhibitor. It simplifies preparation of reverse transcription reaction mixture by adding template RNA and primers without any extra process. Furthermore, by using the RNase H^+ of *M-MLV* Reverse Transcriptase, template RNA is removed after the cDNA synthesis to minimize the effect of residual template RNA in PCR.

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
- Real-time PCR

Features & Benefits

- User-friendly: Reactants are included in a tube, it allows any user simply perform cDNA synthesis by adding template RNA and primers.
- Stability: Included stabilizer and RNase inhibitor provides high resistance to degradation.
- 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.

Composition

Composition	Concentration
M-MLV Reverse Transcriptase	200 U
dNTPs (dATP, dCTP, dGTP, dTTP)	Each 250 μM
5X Reaction buffer	1X
DTT	0.25 mM
Stabilizer	1X

Specifications

M-MLV 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

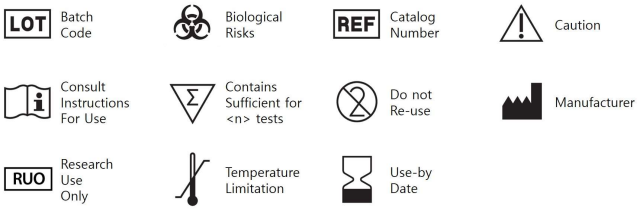
Ordering Information

Description		Cat. No.
1 ml of 2X Master Mix solution	1 ml x 1 ea	K-2263

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

<table>
<tr><th colspan="2">Steps</th><th>Procedure Details</th></tr>
<tr><td>1</td><td> 
Primer annealing</td><td>1. Mix template RNA and primers in a sterile tube (not provided) indicated as below.

• Amount of template RNA and primers
<table><tr><th colspan="2">Components</th><th>20 µl reaction</th><th>50 µl reaction</th></tr><tr><td rowspan="2">Template RNA</td><td>Total RNA</td><td>0.5-1 µg</td><td>1-2 µg</td></tr><tr><td>Poly(A) RNA</td><td>0.05-0.1 µg</td><td>0.1-0.2 µg</td></tr><tr><td rowspan="3">Primers</td><td>Oligo dT</td><td>100 pmol</td><td>200 pmol</td></tr><tr><td>Random primer</td><td>100 pmol</td><td>200 pmol</td></tr><tr><td>Gene specific primer</td><td>10-30 pmol</td><td>20-50 pmol</td></tr></table>
2. Incubate the mixture at 70°C for 5 min and place it on ice directly.</td></tr>
<tr><td>2</td><td> 
Thaw reagents</td><td>3. Thaw AccuPower® RT Master Mix on ice and mix thoroughly before use. Then, briefly spin down components.

4. Dispense appropriate volumes of AccuPower® 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.</td></tr>
<tr><td>3</td><td> 
cDNA synthesis</td><td>5. Transfer the incubated primer annealing mixture to PCR tubes including master mix, and then fill up the nuclease-free water to make a total volume of 20 µl or 50 µl.

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

7. Perform the reaction under the following conditions.
<table><tr><th>Step</th><th>Temperature</th><th>Time</th></tr><tr><td>cDNA synthesis</td><td>42°C</td><td>60 min</td></tr><tr><td>Heat inactivation</td><td>95°C</td><td>5 min</td></tr></table>
8. After the reaction, maintain the reaction mixture at 4°C. The samples can be stored at -20°C until use.</td></tr>
<tr><td></td><td> 
Option</td><td>• If PCR is needed, transfer 2-5 µl of reaction mixture (synthesized cDNA) into AccuPower® PCR PreMix tubes (K-2012, not provided), and perform the reaction under the following conditions.
<table><tr><th>Step</th><th>Temperature</th><th>Time</th><th>Cycles</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 rowspan="3">25-35 cycles</td></tr><tr><td>Annealing</td><td>45-65°C</td><td>20 sec</td></tr><tr><td>Extension</td><td>72°C</td><td>0.5-1 min/kb</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.</td></tr>
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