# *AccuPower*® RT-PCR Master Mix



## [Cat. No.] K-2264

## Introduction

*AccuPower*® RT-PCR Master Mix utilizes one-step RT-PCR performing cDNA synthesis and PCR in a single step, in a single tube. It can provide reduced possibility of cross-contamination and errors. This product contains vacuum-dried components including *M-MLV* Reverse Transcriptase, reaction buffer, RNase inhibitor, *Top* DNA Polymerase, stabilizer, and dNTPs. It simplifies preparation of reverse transcription reaction mixture and PCR 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. After the reaction, samples can be applied directly on agarose gel for analysis due to included tracking dye.

## Applications

- First-strand synthesis of cDNA from RNA molecules (RT)
- RT-PCR
- cDNA library construction
- Gene expression analysis

## Features & Benefits

- Ease-of-use: Reactants are included in a tube, it allows any user simply perform cDNA synthesis and PCR in one tube by adding template RNA and its specific 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 |
| 5X Reaction buffer | 1X |
| DTT | 0.25 mM |
| dNTPs (dATP, dCTP, dGTP, dTTP) | Each 250 μM |
| RNase inhibitor | 1 U |
| *Top* DNA Polymerase | 1 U |
| Stabilizer and tracking dye | 1X |

## Specifications

| *Top* DNA Polymerase | |
|---|---|
| 5' to 3' exonuclease activity | No |
| 3' to 5' exonuclease activity | No |
| 3'–A overhang | Yes |
| Fragment size | Up to 5 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

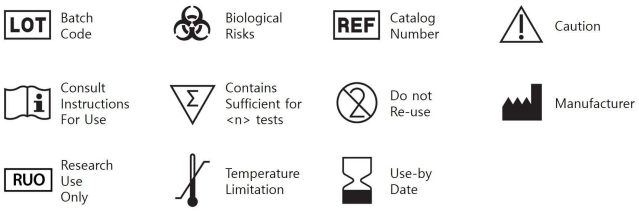
## Ordering Information

| Description | | Cat. No. |
|---|---|---|
| 1 ml of 2X Master Mix solution | 1 ml x 1 ea | K-2264 |

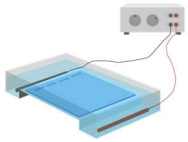
## Notice

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>  <br>Thaw reagents</td><td>1. Thaw AccuPower® RT-PCR Master Mix on ice and mix thoroughly before use. Then, briefly spin down components.<br><br>2. Dispense appropriate volumes of AccuPower® RT-PCR 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>2</td><td>  <br>Preparation of reaction mixture</td><td>3. Add template RNA and primers into PCR tubes including master mix, and then fill up the nuclease-free water to make a total volume of 20 µl or 50 µl.<br><br>• Amount of template RNA and primer<br><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>Primers</td><td>Gene specific primer</td><td>10-30 pmol</td><td>20-50 pmol</td></tr></table><br>4. Mix the reaction mixture by vortexing or pipetting, and briefly spin down.</td></tr>
<tr><td>3</td><td>  <br>RT-PCR</td><td>5. Perform the reaction under the following conditions.<br><table><tr><th>Step</th><th>Temperature</th><th>Time</th><th>Cycles</th></tr><tr><td>cDNA synthesis</td><td>42°C</td><td>60 min</td><td>1 cycle</td></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>10-30 sec</td><td></td></tr><tr><td>Annealing</td><td>45-65°C</td><td>10-30 sec</td><td>30-35 cycles</td></tr><tr><td>Extension</td><td>72°C</td><td>1 kb/min</td><td></td></tr><tr><td>Final extension</td><td>72°C</td><td>5 min</td><td>1 cycle</td></tr></table>* Note: Annealing temperature and time need to be optimized for each primer/template combination.</td></tr>
<tr><td>4</td><td>  <br>Analyze with gel electrophoresis</td><td>6. After the reaction, maintain the reaction mixture at 4°C. The samples can be stored at -20°C until use.<br><br>7. Load samples on agarose gel without adding a loading-dye mixture, and perform gel electrophoresis for analysis.</td></tr>
</table>