# 2X One Step RT-PCR Mix

For research use only

**Cat. No.: RP1001B  Spec.: 50 rxns**

**Components**

| Component | RP1001B (50 rxns, 50 μl/rxn) |
|---|---|
| 2X RT-PCR Reaction Mix [1] | 1.25 ml |
| RT-PCR Enzyme Mix[2] | 125 μl |
| RNase-free dd$H_2O$ | 1 ml |

[1] Contains dNTPs and buffer components；[2] Contains Reverse Transcriptase, RNase inhibitor, hotstart Taq DNA Polymerase, Heat labile DNase.

**Storage Condition**

This product should be kept at -15°C ~ -25°C.

**Description**

The 2X One Step RT-PCR Mix is a one-step kit for reverse transcription and endpoint PCR. It amplifies RNA target sequences with gene-specific primers (GSP). Optimized buffer components enable reverse transcription and PCR reactions to be performed within one system. This product is composed of a two-fold concentration of reaction buffer and an enzyme mixture, which is more conducive to the stable preservation of enzyme reagents than a one-tube all-in mix. This product is simple and convenient to use, only GSP and template RNA need to be added to use, and no additional capping and pipetting operations are required, which not only saves time, but also effectively reduces the risk of contamination. This product contains heat labile DNase, which can remove genomic DNA at the same time when reverse transcription. Heat labile DNase is susceptible to high temperature inactivation and is conducive to stable preservation of cDNA or amplification reactions.

**Protocol Example**

**1. Preparation of reaction solution**

1.1 Prepare the reaction system as follows. Melt all reagents on ice. When preparing multiple reaction wells, leave a 10% margin for each component to avoid pipetting loss.

| Component | Volume | Final Conc. |
|---|---|---|
| 2X One Step RT-PCR Mix | 25 μl | 1X |
| RT-PCR Enzyme Mix | 2.5 μl | - |
| Forward Primer (10 μM) | 2 μl | 0.4 μM |
| Reverse Primer (10 μM) | 2 μl | 0.4 μM |
| Template RNA | Variable | 1 pg-1 μg |
| RNase-free dd$H_2O$ | Variable | - |
| Total volume | 50 μl | - |

1.2 After the reaction system is ready, fully flip and mix well, and centrifuge briefly.

**2. Perform RT-PCR**

Fast RT-PCR mode (sequences ≤ 2 kb):

| Step | Stage | Cycle | Temperature | Time |
|---|---|---|---|---|
| Reverse transcription | 1 | 1 | 50°C [1] | 30 min |
| Initial denaturation | 2 | 1 | 95°C | 2 min |
| Denaturation | 3 | 30~35 | 95°C | 30 sec |
| Annealing | | | 55~68°C [2] | 30 sec |
| Entension | | | 72°C | Set the time by the speed of 0.5 min/kb |
| Final extension | 4 | 1 | 72°C | 5~10 min |

Standard RT-PCR mode (sequences > 2 kb):

| Step | Stage | Cycle | Temperature | Time |
|---|---|---|---|---|
| Reverse transcription | 1 | 1 | 50°C [1] | 30 min |
| Initial denaturation | 2 | 1 | 95°C | 2 min |
| Denaturation | 3 | 30~35 | 95°C | 30 sec |
| Annealing | | | 55~68°C [2] | 60 sec |
| Entension | | | 72°C | Set the time by the speed of 1 min/kb |
| Final extension | 4 | 1 | 72°C | 5~10 min |

1. The temperature of the reverse transcription reaction can be adjusted between 48°C and 55°C. For templates with complex secondary structures or high GC regions, increasing the reverse transcription temperature to 55ºC is conducive to improving amplification efficiency and sensitivity.

2. The denaturation temperature is set to around the primer Tm-5℃.

**3. Analyze The Results**

Detecte and analyze the reaction products by agarose gel electrophoresis.

**Notes**

1. During the operation, pay attention to prevent RNase contamination, wear a clean mask and gloves, and use consumables that are RNase-free.
2. Use high-quality RNA as a template. The presence of degraded RNA, RNase and other impurities will affect the efficiency of reverse transcription.
3. The primer length should be designed between 18-30 bases and the GC content should be between 40 and 60%.

**Product Use Limitations**

This product is sold exclusively for research purposes and *in vitro* use. Neither the product, nor any individual components, was tested for use in diagnostic applications or for drug development, nor is it suitable for administration to humans or animals. Please refer to the MSDS, available upon request.