# Operation Guide of FastKing RT Kit (With gDNase) (KR116)

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Version No.: 20170420

# Experiment Preparation

1. RNA sample
2. Pipette and matched sterile tips (RNase-free)
3. 1.5 ml centrifuge tube (RNase-free) and 200 μl PCR tube (RNase-free)
4. Vortex oscillator, centrifuge, dry bath/thermal cycler

# Step 1



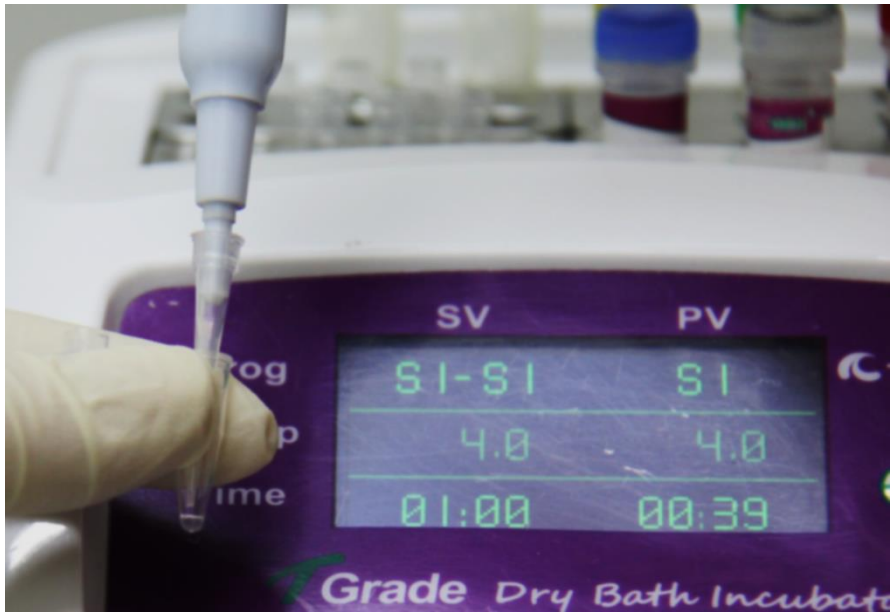

Thaw the template RNA on ice; place the FastKing RT Enzyme Mix on ice; thaw other components at room temperature and quickly place on ice after thawing. Before use, mix each solution evenly by vortex, and briefly centrifuge to collect the liquid remaining on the tube wall.

# Step 2

Prepare the genomic DNA removal reaction mixture under the ice bath conditions in accordance with the gDNA removal system in Table 1, and then mix them evenly. Incubate for 3 min at 42℃ after a brief centrifugation, and then put it on the ice.

Table 1 gDNA Removal Reaction System

| Component | Volume |
|---|---|
| 5×gDNA Buffer | 2 μl |
| Total RNA | 50 ng-2 μg |
| RNase-Free dd$H_2O$ | Up to 10 μl |

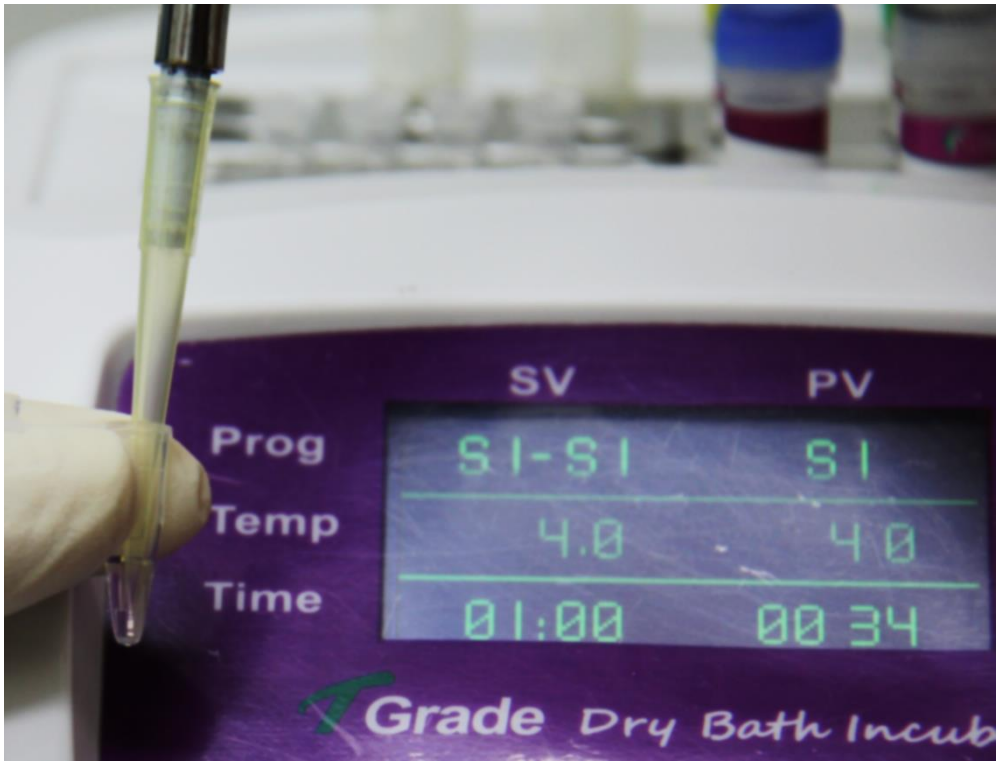


# Step 3

Prepare the reverse transcription reaction mixture under the ice bath conditions in accordance with the reverse transcription reaction system in Table 2.

Table 2 Reverse Transcription Reaction System

| Component | Volume |
|---|---|
| 10×King RT Buffer | 2 μl |
| FastKing RT Enzyme Mix | 1 μl |
| FQ-RT Primer Mix | 2 μl |
| RNase-Free dd$H_2O$ | 5 μl |
| Total volume | 10 μl |



# Tips

1. When preparing the reverse transcription mixture according to the reverse transcription reaction system in Table 2, the required reaction quantity shall be determined first. Calculate the total volume of reagents required and increase the volume by 10%-20% to compensate for the pipetting loss, thus to ensure the solution is sufficient for desired numbers of reactions. For example, when a total of 5 reverse transcription reactions are required, the number of system preparations is at least 6; when a total of 10 reverse transcription reactions are required, the number of system preparations is at least 11; when a total of 20 reverse transcription reactions are required, the number of system preparations is at least 22. And so on.
2. The required amount of each component is calculated according to the number of reactions. Prepare all components into the same tube on ice, thoroughly mix, and centrifuge for a short time.

| Reagent | Volume of 1 system | Volume of 6 systems | Volume of 11 systems | Volume of 22 systems |
|---|---|---|---|---|
| 10×King RT Buffer | 2 μl | 12 μl | 22 μl | 44 μl |
| FastKing RT Enzyme Mix | 1 μl | 6 μl | 11 μl | 22 μl |
| FQ-RT Primer Mix | 2 μl | 12 μl | 22 μl | 44 μl |
| RNase-Free dd$H_2O$ | 5 μl | 30 μl | 55 μl | 110 μl |
| Total volume | 10 μl | 60 μl | 110 μl | 220 μl |

# Step 4

Add 10 μl reverse transcription mixture in each gDNA removal mixture (10 μl), and then mix thoroughly to form a 20 μl reaction system.

# Step 5


Incubate for 15 min at 42℃.

# Step 6


Incubate for 3 min at 95℃ and then put on ice to complete the experiment.

# Tips


The obtained cDNA can be used for subsequent experiments or cryopreservation. Divide it into single-use aliquots and avoid repeated freezing and thawing.

It's not suggested to detect the cDNA concentration.