Operation Guide of FastKing One Step RT-PCR Kit (KR123)

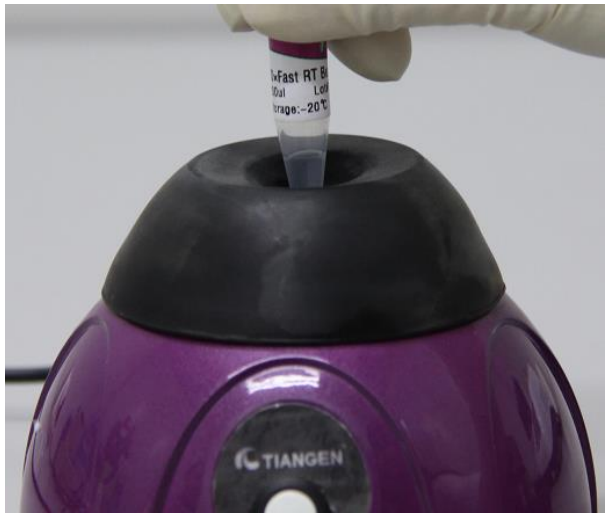
TIANGEN BIOTECH(BEIJING)CO., LTD

Version No.: 20170424

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, specific primer, 2×FastKing One Step RT-PCR MasterMix and RNase-Free ddH_2O completely and then place on ice bath after a short centrifugation.

Step 2

Prepare the reaction solution under the ice bath conditions according to the following table.

Component	Volume/reaction
2×FastKing One Step RT-PCR MasterMix	25 µl
25×RT-PCR Enzyme Mix	2 µl
Forward primer (10 µM)	1.25 µl
Reverse primer (10 µM)	1.25 µl
RNA template	10 ng-1 µg total RNA
RNase-Free ddH_2O	Up to 50 µl
Total system	50 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	Usage amount of 1 system	Usage amount of 6 systems	Usage amount of 11 systems	Usage amount of 22 systems
2×FastKing One Step RT-PCR MasterMix	25 μl	150 μl	275 μl	550 μl
25×RT-PCR Enzyme Mix	2 μl	12 μl	22 μl	44 μl
Upstream specific primer (10 μM)	1.25 μl	7.5 μl	13.75 μl	27.5 μl
Downstream specific primer (10 μM)	1.25 μl	7.5 μl	13.75 μl	27.5 μl
Total volume of mixture	29.5 μl	177 μl	324.5 μl	649 μl

3. Calculate the required volume of RNA template and ddH_2O to be added per sample.
4. The system shall be prepared by the sequence of Mixture-RNA-ddH_2O, and thoroughly mixed.

Step 3

Set PCR reaction conditions according to the following table:

Reaction temperature	Reaction time	Number of cycles	Description
42°C	30 min	1	Reverse transcription
95°C	3 min	1	Initial denaturation
94°C	30 sec	35-40	PCR cycles
55-65°C	30 sec		
72°C	30 sec		
72°C	5 min	1	Final extension

Tips

In order to avoid non-specific amplification, the preparation of one-step reaction solution should always be carried out in ice bath, and put the reaction tubes into the thermal cycler when the instrument temperature reaches 42℃.

The annealing temperature can be adjusted according to different primers.

Step 4

Detect and analyze the PCR products by agarose gel electrophoresis.