RT-PCR Mix for qPCR

For research use only

Cat. No.: R1031 **Spec.: 100 rxns**

Components

Component	R1031 (100 rxns, 20 μl/rxn)
RT-PCR Enzyme Mix	100 μl
5X RT-PCR Buffer	400 μl
Control Mix	20 μl
RNase-free ddH_2O	1 ml × 2

Storage

All components should be stored at -20°C, and avoid repeated freezing and thawing.

Description

RT-PCR Mix for qPCR is a premixed reverse transcription reaction (RT-PCR) kit, easy to operate, can quickly and efficiently complete the reverse transcription reaction. RT-PCR Enzyme Mix contains highly efficient reverse transcriptase and heat labile DNase, which can complete genomic DNA clearance and RT-PCR of RNA in one step. 5X RT-PCR Buffer contains other components required for RT-PCR, without the need for multiple addition of components step by step, thus reducing the risk of sample contamination. At the same time, the kit is equipped with Control Mix for a negative control reaction to test for genomic DNA residue in the RNA template. The reverse transcription products of this kit have good compatibility for real-time PCR (qPCR) reaction by dye method and probe method, and can be used for accurate gene expression analysis.

Application

First strand cDNA synthesis.

Important notes

1. This product contains heat labile DNase, so please use each component on ice.
2. To ensure uniform composition, please centrifuge briefly of RT-PCREnzyme Mix, 5X RT-PCR Buffer and Control Mix, then use pipette to blow and mix well before use.
3. During the operation, care should be taken to prevent RNA degradation due to the introduction of RNase. RNA is best stored at -70°C, and avoid repeated freeze-thaw.
4. cDNA products should be stored at -20°C or below, and repeated freeze-thaw should be avoided.

Protocol

1. Prepare the reaction system

Prepare the reaction mixture in an RNAse-free centrifuge tube according to the following system:

Component	Amount
5X RT-PCR Buffer	4 μl
RT-PCREnzyme Mix	1 μl
Template RNA	1pg-1μg
RNase-free ddH_2O	To 20 μl

Negative control reaction (optional)

That is, without the addition of reverse transcriptase, the product is used as a negative control in qPCR to test whether there is genomic DNA residue in template RNA.

Prepare the reaction mixture in an RNAse-free centrifuge tube according to the following system:

Component	Amount
5X RT-PCR Buffer	4 μl
Control Mix	1 μl
Template RNA	1pg-1μg
RNase-free ddH_2O	To 20 μl

2. RT-PCR

Blow and mix the above mixture with pipette, then collect by instantaneous centrifugation to the bottom of the tube.

Run the reaction in the PCR instrument according to the following procedure:

Temperature	Time
50°C	15 min
85°C	5 sec

3. qPCR and Storage of cDNA

The diluted cDNA products can be immediately used to dye or probe qPCR reaction. If undiluted cDNA stock is used, the volume should not exceed 1/10 of the reaction system to avoid inhibiting the reaction efficiency.

Long-term storage of cDNA products should be kept at -20° C or below, and repeated freezing and thawing should be avoided.