

# FastKing One Step RT-PCR Kit

---

For fast and sensitive one-step RT-PCR

[www.tiagen.com/en](http://www.tiagen.com/en)

This product is for scientific research use only. Do not use in  
medicine, clinical treatment, food or cosmetics.

# FastKing One Step RT-PCR Kit

Cat. no. 4992294

## Kit Contents

Contents	4992294 50 $\mu$ l $\times$ 50 rxn
2 $\times$ FastKing One Step RT-PCR MasterMix	1.25 ml
25 $\times$ RT-PCR Enzyme Mix	100 $\mu$ l
RNase-Free ddH <sub>2</sub> O	2 $\times$ 1 ml
Handbook	1

## Storage

Store at -30~-15°C for up to one year.

## Introduction

FastKing One Step RT-PCR Kit allows both reverse transcription and gene amplification to take place in a single tube, which avoids cross contamination between samples and improves the sensitivity of detection. The 25×RT-PCR Enzyme Mix contains King reverse transcriptase, which is a high efficient reverse transcriptase expressed by engineering bacteria; a further-modified hot start Taq DNA polymerase, which provides high efficiency and accuracy for the amplify reaction; and RNase inhibitor. With a special modified hydrophobic motif, King RTase gets a significant affinity for RNA and facilitates transcription through of RNA templates, especially for templates with high GC content or complex secondary structures. The 2×FastKing One Step RT-PCR MasterMix contains appropriate ion concentration, dNTPs and PCR enhancer. It could stabilize both RTase and polymerases and keep their efficiency within the whole reaction process.

## Materials required but not supplied

1. RNA templates
2. gene-specific PCR primers

## Protocol

1. Thoroughly thaw the template RNA, gene-specific PCR primers, 2×FastKing One Step RT-PCR MasterMix and RNase-Free ddH<sub>2</sub>O, centrifuge briefly and place them on ice.
2. Prepare a reaction solution according to the following table on ice:

Contents	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
Template RNA	10 ng-1 μg total RNA
RNase-Free ddH <sub>2</sub> O	Up to 50 μl

**Notes: If setting up more than one RT-PCR reaction, mix all components one time and divide into each tube.**

3. Set up thermal cycler conditions according the following table.

Steps	Reaction	Time	Temperature
1	Reverse transcription	30 min	42°C
2	Initial denaturation	3 min	95°C
3	Denaturation	30 sec	94°C
4	Annealing	30 sec	55-65°C
5	Extension	1 kb/min	72°C
6	35-40 cycles from step 3 to step 5		
7	Final extension	5 min	72°C

**Notes: To avoid unspecific amplification, start the RT-PCR program while PCR tubes are still on ice. Wait until the thermal cycler has reached 42°C. Then place the PCR tubes in the thermal cycler. The annealing temperature depends on different primers.**

4. Analyze the PCR products using agarose gel electrophoresis.

### Important Notes

1. RNA template could be total RNA or mRNA. TRNzol or RNAprep pure kits can be used to purify high-quality total RNA.
2. RNase contaminations should be avoided in one-step RT-PCR. Some measures can be taken as below:
  - 1) Wear a disposable gloves and respirator to avoid the RNase contaminations from skin and saliva
  - 2) Operate the RNA related experiments in an RNase-free environment using RNase-free apparatus and consumable items.
  - 3) Consumable items related with RT-PCR should be incubated in 0.1% DEPC solution at 37°C for 12 hours and sterilized for 30 min before use.
3. 25×RT-PCR Enzyme Mix should be centrifuged briefly before use. Pipet slowly and store at -30~-15°C immediately after use.
4. Completely mix the 2×FastKing One Step RT-PCR MasterMix and centrifuge the reagent to the botiom of the tube before use.
5. Only use gene-specific primers and use specific primers according to specific requirements of experiments.