AccurSTART One Step RT-PCR Kit

P613

Version 24.1

Product Description

AccurSTART One Step RT-PCR Kit is a one-step RT-PCR reagent kit for detection using RNA as a template. This kit has been optimized to achieve a one-tube operation for reverse transcriptase, DNA polymerase, and buffer. The upgraded reverse transcriptase and DNA polymerase combination enhances the specificity, yield, and sensitivity of the reagents. The reagent is equipped with 5 × gDNA Wiper Components, further purifying RNA samples, resulting in more accurate and reliable amplification results. AccurSTART One Step RT-PCR Kit can detect total RNA as low as 0.1 pg, stably detecting fragments up to 10 kb, and is compatible with multiplex amplification systems, ensuring high yield and high sensitivity of the RT-PCR reaction.

Components

	Components	P613-01 50 rxns (50 µl/rxn)
■	5 × gDNA Wiper Mix[a]	200 µl
■	2 × One Step RT-PCR Master Mix[b]	1.25 ml
□	10 × DNA Loading Buffer[c]	1.25 ml

a. It contains DNase I, etc.

b. It contains Reaction Buffer, Reverse Transcriptase, RNase Inhibitor, and Taq DNA Polymerase Pro.

c. It contains bromothymol blue, etc.

Storage

Store at -30 ~ -15°C and ship at ≤0°C.

Applications

It is applicable for animals, plants, microorganisms (viruses, etc.) various RNA nucleic acid testing.

Notes

Prevent RNase contamination, please keep the experimental area clean; during operation, it is necessary to wear clean gloves and masks; all consumables used in the experiment, such as centrifuge tubes and pipette tips, must be guaranteed to be RNase-free.

Experiment Process

◇RNA template in genome elimination (optional)

Prepare the following reaction solution in RNase-free centrifuge tubes:

Components	Volume	
RNase-free ddH_2O	to 20 µl	
5 × gDNA Wiper Mix	4 µl	■
Template RNA	Total RNA: 0.1 pg - 1 µg	

Use a pipette to gently pipette up and down several times to mix thoroughly or briefly vortex and then centrifuge at 42°C for 2 min.

▲ Do not pre-mix the Components and Primer.

▲ This step is optional; if there is no need to remove genomic DNA, this step can be omitted directly.

◇Prepare RT-PCR reaction system

Components	Volume	
2 × One Step RT-PCR Master Mix	25 µl	■
Primer Forward (10 µM)	2 µl	
Primer Reverse (10 µM)	2 µl	
Template RNA	Total RNA: 0.1 pg - 1 µg	
RNase-free ddH_2O	to 50 µl	

▲ The digested template can add up to 20 µl to the reaction system.

▲ The reaction volume can be adjusted according to experimental needs, and the amounts of each component only need to be adjusted in proportion.

◇Conduct one-step RT-PCR reaction under the following conditions

Standard program

a. Target fragment <5 kb

Step	Temperature	Time	Cycles
Reverse Transcription	55°C[a]	30 min	
Initial Denaturation	94°C	3 min	
Denaturation	94°C	30 sec	30 - 35[d]
Annealing	60°C[b]	30 sec	
Extension	72°C	0.5 - 1 min/kb[c]	
Final Extension	72°C	5 min	

b. Target fragment >5 kb

Step	Temperature	Time	Cycles
Reverse Transcription	55°C[a]	30 min	
Initial Denaturation	94°C	3 min	
Denaturation	94°C	30 sec	30 - 35[d]
Extension	68°C[b]	1 min/kb[c]	
Final Extension	72°C	5 min	

a. For templates with complex secondary structures or high GC regions, 55°C reverse transcription can improve amplification efficiency and sensitivity.

b. Annealing temperature is recommended to be set in the range of 50 ~ 60°C, generally set to be 5°C lower than the Primer Annealing temperature; for fragments >5 kb, it is recommended to use long Primers with Tm values in the range of 68 ~ 70°C, combining the Annealing/Extension temperature to 68°C, which can significantly improve amplification specificity.

c. For fragments <5 kb, the Extension time should be set to at least 0.5 min/kb; for fragments >5 kb, the Extension time should be set to at least 1 min/kb. Generally speaking, extending the Extension time is beneficial for increasing amplification yield.

d. Cycles can be adjusted according to actual conditions. When gene expression levels are low or template concentration is low, Cycles can be appropriately increased to enhance amplification yield.

Fast program

purpose fragment <5 kb

Step	Temperature	Time	Cycles
Reverse Transcription	55°C	5 min	
Initial Denaturation	94°C	3 min	
Denaturation	94°C	15 sec	30 - 35
Annealing	60°C	30 sec	
Extension	72°C	30 sec/kb	
Final Extension	72°C	5 min	

▲ Fast program reaction time at each stage can be adjusted according to individual needs.

◇Check product by agarose gel electrophoresis

1. Refer to the table below to prepare the reaction system, add an appropriate amount of 10 × DNA Loading Buffer to a final concentration of 0.5 - 1 ×.

Components	Volume
10 × DNA Loading Buffer	2.5 - 5 μl
DNA Sample	x μl
ddH_2O	to 50 μl

▲ The reaction volume can be adjusted according to experimental needs, and the amounts of each component only need to be adjusted in proportion.

2. Gently mix, briefly centrifuge to collect at the bottom of the tube, take an appropriate amount for agarose gel electrophoresis.