AccurSTART One Step RT-PCR Kit (Dye Plus)

P614

Version 24.1

Product Description

AccurSTART One Step RT-PCR Kit (Dye Plus) is a one-step reverse transcription PCR reagent kit designed for the detection of RNA targets. This kit is optimized for one-tube operation, integrating reverse transcriptase, DNA polymerase, and buffer into a single reaction system. The enhanced reverse transcriptase and DNA polymerase combination improves the specificity, yield, and sensitivity of the reaction. The reagent contains a red tracking dye, allowing direct gel loading following the reaction, thereby enhancing convenience and efficiency. The kit is equipped with 5 × gDNA Wiper components, which further purify RNA samples and ensure more accurate and reliable amplification results. The One Step RT-PCR Kit is capable of detecting as little as 0.1 pg of total RNA, stably amplifying fragments up to 10 kb, and is compatible with multiplex amplification systems, thereby ensuring high yield and sensitivity of the RT-PCR reactions.

Components

	Components	P614-01 50 rxns (50 µl/rxn)
■	5 × gDNA Wiper Mix[a]	200 µl
■	2 × One Step RT-PCR Master Mix (Dye Plus)[b]	1.25 ml

a. It contains DNase I, etc.

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

Storage

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

Applications

It is applicable to RNA nucleic acid testing in animals, plants and microorganisms (including viruses, etc.).

Notes

To prevent RNase contamination, maintain a clean experimental environment; wear clean gloves and a mask during the procedure, and ensure that all consumables, including centrifuge tubes and pipette tips, are RNase-free.

Experiment Process

◇ RNA template from 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	

Mix the sample thoroughly by gently pipetting up and down several times, or by briefly vortexing and then centrifuging. Incubate at 42°C for 2 min.

▲ Do not pre-mix the components and primer.

▲ This step is optional, if genomic DNA does not need to be removed, the step may be omitted.

◇ Prepare RT-PCR reaction system

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

Components	Volume
2 × One Step RT-PCR Master Mix (Dye Plus)	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 be added up to 20 μl to the reaction system.

▲ The reaction volume can be adjusted according to experimental requirements, and the amounts of each component should be adjusted proportionally.

◇ Perform a one-step RT-PCR reaction under the following conditions

Standard program

1. 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	

2. 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. Reverse transcription at 55°C is recommended for templates with complex secondary structures or high GC content, to 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.

◇ Directly check the product by agarose gel electrophoresis