HiScript II One Step RT-PCR Kit

Catalog # P611

Version 7.1

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Introduction

The Vazyme HiScript II One Step RT-PCR Kit is specially designed for RNA detection (such as RNA virus). With the HiScript II One Step RT-PCR Kit and gene-specific primers (GSP), both reverse transcription and PCR amplification are performed in the same tube, with no additional pipetting procedures, which improves detection through-put and minimizes potential contamination. This kit contains HiScript II Reverse Transcriptase, Champagne Taq plus hot-start DNA Polymerase, and an optimized buffer, which enables high-sensitive total RNA detection and long-fragment amplification (as long as 10 kb).

Contents of Kits

Components	P611-01 125 rxn (20 μl/rxn)
RNase free ddH_2O	1 ml × 2
2× One Step Mix [a]	625 μl × 2
One Step Enzyme Mix [b]	125 μl
10× Loading buffer	1.25 ml

a. Contains dNTPs.
b. Contains RNase inhibitor, HiScript II Reverse Transcriptase, and Champagne Taq plus Polymerase.

Storage

All components should be stored at -20℃.

Protocol

Note: To avoid RNase contamination, please keep the experiment area clean, wear clean gloves and masks, and use RNase-free tubes and tips.

1. Mix the following components in a RNase-free PCR tube:

RNase free ddH_2O	to 20 μl
2× One Step Mix	10 μl
One Step Enzyme Mix	1 μl
Gene Specific Primer Forward (10 μM)	0.8 μl
Gene Specific Primer Reverse (10 μM)	0.8 μl
Template RNA	Total RNA: 0.1 pg-0.5 μg

2. Put the tube into a thermocycler and run the following program:

For fragments ≤ 5 kb (3-Step PCR)

50℃ [a]	30 min	
94℃	3 min	
94℃	30 sec	30-35 cycles
55℃-72℃ [b]	30 sec	
72℃	0.5-1 min/kb [c]	
72℃	5 min	
4℃	Hold	

For fragments > 5 kb (2-Step PCR)

50℃ [a]	30 min	
94℃	3 min	
94℃	10 sec	30-35 cycles
68℃ [b]	1 min/kb [c]	
72℃	5 min	
4℃	Hold	

Note: a. For templates with complex secondary structure or high GC-content, the temperature can be increased to 55℃, which will benefit the yield.

b. The temperature for annealing is usually 1-2℃ low than the Tm of the primers. For fragments > 5 kb, a 2-step PCR program is recommended to significantly improve the specificity, which use longer primers and combines annealing and extension into one step.

c. Longer extension time is helpful to increase the amplification yield.

3. Evaluate the PCR products via agarose gel electrophoresis.