

HiScript II One Step RT-PCR Kit (Dye Plus)

Catalog # P612



Version 7.1

Vazyme biotech co., ltd.

Introduction

The Vazyme HiScript II One Step RT-PCR Kit (Dye Plus) 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, RNase inhibitor, and Champagne Taq plus hot-start DNA Polymerase, which enables high-sensitive total RNA detection (as little as 1 pg) and long-fragment amplification (as long as 10 kb). The 2× One Step Mix (Dye Plus) contains an optimized buffer, dNTPs, and Loading Dye.

Contents of Kits

Components	P612-01 50 rxn (50 µl/rxn)
RNase free ddH ₂ O	1 ml × 2
2× One Step Mix (Dye Plus) ^a	625 µl × 2
One Step Enzyme Mix ^b	125 µl

a. Contains dNTPs and Loading Dye.

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

Storage

All components should be stored at -20°C.

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 ₂ O	to 50 µl
2× One Step Mix (Dye Plus)	25 µl
One Step Enzyme Mix	2.5 µl
Gene Specific Primer Forward (10 µM)	2 µl
Gene Specific Primer Reverse (10 µM)	2 µl
Template RNA	Total RNA: 0.1 pg-1 µg

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

For fragments ≤ 5 kb (3-Step PCR)

50°C ^a	30 min	} 30-35 cycles
94°C	3 min	
94°C	30 sec	
55°C-72°C ^b	30 sec	
72°C	0.5-1 min/kb ^c	
72°C	5 min	
4°C	Hold	

For fragments > 5 kb (2-Step PCR)

50°C ^a	30 min	} 30-35 cycles
94°C	3 min	
94°C	10 sec	
68°C ^b	1 min/kb ^c	
72°C	5 min	
4°C	Hold	

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

b. The temperature for annealing is usually 1-2°C low than the T_m 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.



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