HiScript II One Step RT-PCR Kit

P611

Version 22.2

Product Description

HiScript II One Step RT-PCR Kit is specially designed for end-point PCR detection that directly use RNA (e.g. virus RNA) as template. Reverse transcription and PCR can be finished in one tube in the presence of Gene Specific Primers (GSP), which requirng no additional opening/pitting operations. This can greatly increase the assay throughput and reduce the risk of contamination. Integrating the superior performance of HiScript II Reverse Transcriptase, Champagne Taq plus DNA Polymerase (dedicated for long fragments), and optimized buffer, this kit can amplify long fragments of more than 10 kb. The kit is provided in the form of Master Mix. 2 × One Step Mix includes optimized buffer system and dNTPs. One Step Enzyme Mix contains proportionally optimized HiScript II Reverse Transcriptase, RNase inhibitor and Champagne Taq plus DNA Polymerase.

Components

	Components	P611-01 50 rxns (50 μl/rxn)
☐	RNase-free ddH_2O	2 × 1 ml
■	2 × One Step Mix[a]	2 × 625 μl
■	One Step Enzyme Mix[b]	125 μl
■	10 × DNA Loading buffer	1.25 ml

a. It contains dNTPs.
b. It contains RNase inhibitor, HiScript II Reverse Transcriptase, Champagne Taq plus DNA Polymerase.

Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

Applications

It is applicable for amplification reaction of animal, plant and microbial RNA.

Notes

For research use only. Not for use in diagnostic procedures.
To avoid RNase contamination, please keep the experimental area clean; clean gloves and masks should be worn during experiment; tubes and tips used in the experiment must be RNase-free.

Experiment Process

1. Mix the following components in an RNase-free centrifuge tube:

RNase-free ddH_2O	to 50 μl	☐
2 × One Step Mix	25 μl	■
One Step Enzyme Mix	2.5 μl	■
Primer 1 (10 μM)	2 μl	
Primer 2 (10 μM)	2 μl	
Template RNA*	Total RNA: 1 pg - 1 μg	

▲ The reaction volume can be adjusted accordingly, the reaction volume can be adjusted accordingly, and the dosage of each component can be adjusted corrspondingly into the same proportion.
* The denaturation of RNA template devotes to eliminating the secondary structure, which can greatly improve the yield of cDNA. For cDNA fragments >5 kb, the template RNA should be denatured before the reaction: Incubate at 65℃ for 5 min, then immediately place on ice for 2 min.

2. Perform One Step RT-PCR reaction as follows:

For fragments ≤5 kb

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

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

a. For template with complex secondary structure or high GC-content, the temperature can be increased to 55℃, which may increase the yield of reverse transcription

b. The temperature for annealing is usually 1 ~ 2℃ lower than the Tm of the primers. For fragments >5 kb, it is recommended to use long primers with Tm values between 68 ~ 70℃, and merge annealing and extention into one step and set the temperature at 68℃, which can significantly improve the amplification specificity.

c. For fragments <5 kb, extension time should be set to ≥20 sec/kb; for fragments >5 kb, extension time should be set to ≥1 min/kb. Generally speaking, longer extension time is helpful to increase the yield of amplification.

3. Evaluate the PCR products by agarose gel electrophoresis.