HiScript® II One Step RT-PCR Kit

P611-01 50

www.yh-biosearch.com
SHANGHAI YEHUA Biological Technology Co., Ltd.

Instructions

Version 1.2

Catalog

1/Product introduction

HiScript® II One Step RT-PCR Kit is specifically designed for PCR detection of end-point method by taking RNA as template (such as, RNA virus), which can finish gene special primer (GSP), reverse transcription and PCR reaction in one pipe without extra operation of opening pipe or liquid relief, and thus greatly improve the detection flux and reduce the risk of pollution.
After integrating with superior capacity of HiScript® II Reverse Transcriptase and Champagne Taq™ plus DNA polymerase, and coordinating with optimized buffering system, the detection sensitivity of HiScript® II One Step RT-PCR Kit can arrive to 0.1pg of total RNA and a fragment with 12kb can be amplified. Moreover, the kit is supplied by a convenient form of Master Mix. 2 × One Step Mix including optimized buffering system and Dntp, while One Step Enzyme Mix contains HiScript® II Reverse Transcriptase, RNase inhibitor and Champagne TaqTM plus DNA Polymerase with optimized scale.

2/Product composition

composition	P611-01 50 rxn (50 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.Including dNTP
b.Including RNase inhibitor

3/Storage condition

Be stored at -20℃.

4/Quality control

According to detection, all compositions do not contain residue of exonuclease, endonuclease and RNase.

Functional test 1： Amplify UTRN gene by taking 1 μg HeLa cell total RNA as template. Please refer to 12.1kb target bands for agarose gel electrophoresis and EB dyeing.
Functional test 2： Amplify GAPDH gene by taking 0.1 pg HeLa cell total RNA as template. Single 550 bp band can be seen for agarose gel electrophoresis and EB dyeing.

5/Precautions

In order to prevent RNase pollution，please keep experimental area clean!
Clean gloves and mask should be put on during operation;
Centrifuge tube, axygen and other consumables used for experiment should be free of RNase.

Application example

1. Configure following mixed liquor in RNase free centrifuge tube:

RNase free ddH_2O	to 50 μl
2 × One Step Mix	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

Note: Adjust reaction volume according to the needs of experiment, and the amount of each composition can be just adjusted according to the relative scale.

2. Conduct One Step RT-PCR reaction according to following conditions:

Target fragment < 5 kb

50°C [a]	30 min	
94°C	3 min	
94°C	30 sec	
55°C~72°C [b]	30 sec [c]	} 30-35 cycles
72°C	0.5-1 min/kb	
72°C	7 min	
4°C	Hold	

Target fragment > 5 kb

50°C [a]	30 min	
94°C	3 min	
94°C	10 sec	
68°C [b]	1 min/kb [c]	} 30-35 cycles
72°C	7 min	
4°C	Hold	

a. If the template has complicated two grade structure or high GC area, you can increase the reaction temperature to 55°C, which will be helpful to improving the output.

b. Annealing temperature should be adjusted according to the annealing temperature of primer, which is usually set as 1-2°C lower than that of primer. For fragment longer than 5kb, it is suggested to use long primer with Tm value in the scope of 68-70°C, and consolidate the annealing/elongation temperature to be 68°C. In this case, the specificity of amplification can be obviously increased.

c. For fragment less than 5 kb, the elongation time should be set as 0.5 min/kb at least. But for fragment longer than 5 kb, the elongation time should be 1 min/kb at lowest. Generally, the extension of elongation is good to improve the output of amplification.

3. Product should be inspected by agarose gel electrophoresis.

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