ACE Scipt II One Step RT-PCR Kit

Cat# EP2006

Storage: All components should be stored at -20℃.

INTRODUCTION

The ACE Scipt II One Step RT-PCR Kit is specially designed for RNA detection (such as RNA virus). With the ACE Scipt 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

Component	EP2006 50 rxn (50 µl/rxn)
RNase free ddH2O	1 ml × 2
2× One Step Mix[a]	625µl × 2
One Step Enzyme Mix[b]	125µl
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 ddH2O	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

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	

PRIMERS DESIGNING NOTES

1. Choose C or G as the last base of the 3'-end of the primer;
2. Avoid continuous mismatching at the last 8 bases of the 3'-end of the primer;
3. Avoid hairpin structure at the 3'-end of the primer;
4. Tm of the primers should be within the range of 55℃ - 65℃;
5. Additional sequence should not be included when calculating Tm of the primers;
6. GC content of the primers should be within the range of 40% - 60%;
7. Tm and GC content of forward and reverse primes should be as similar as possible.

PRODUCT USE LIMITATION

These products are intended for research use only.