TransScript® II Two-Step RT-PCR SuperMix

Please read the manual carefully before use.

Cat. No. AH401

Version No. Version 1.0

Storage: at -20℃ for two years

Description

TransScript® II Two-Step RT-PCR SuperMix is a two-step RT-PCR kit with high synthesis capacity and high amplification efficiency. The first-strand cDNA is synthesized efficiently from RNA by reverse transcription with 5×*TransScript*® II All-in-One SuperMix for PCR at 42°C-55°C; and amplified by PCR with 2×*TransTaq*® HiFi PCR SuperMix II. This kit contains all the reagents for reactions from RNA to cDNA and PCR amplification for this cDNA.

Features

- Highly efficient synthesis of the first-strand cDNA from RNA with 5×*TransScript*® II All-in-One SuperMix for PCR, allowing for easy operation and minimal contamination during the operation.
- 2×*TransTaq*® HiFi PCR SuperMix II is suitable for cDNA amplification, featuring high fidelity, high specificity, and high amplification efficiency.
- The amplified product has an "A" base at the 3' end and can be directly cloned into the *pEASY*®-T series vector.
- Amplification of fragment up to 15 kb.

Applications

- cDNA library construction, 3' and 5' RACE.
- Multiple copy and low copy gene detection.
- RNA template with GC-rich or complex secondary structure.

Kit Contents

Component	AH401-01
5×*TransScript*® II All-in-One SuperMix for PCR	200 µl
2×*TransTaq*® HiFi PCR SuperMix II	2×1 ml
RNase-free Water	1 ml

Before use, please centrifuge each component briefly.

First-strand cDNA synthesis

1. Prepare reaction mix wth the following components

Component	Volume
Total RNA/mRNA	0.1 ng-5 µg/10 pg-500 ng
5×*TransScript*® II All-in-One SuperMix for PCR	4 µl
RNase-free Water	Variable
Total volume	20 µl

2. Mix well gently for reverse transcription

- For RNA template with poly(A)+, incubate at 50°C for 30 minutes.
- For RNA template without poly(A)+, incubate at 25°C for 10 minutes, then at 50°C for 30 minutes.
- For GC-rich or complex secondary structure RNA template, incubate at 55°C for 30 minutes.

3. Incubate at 85°C for 5 seconds to inactivate *TransScript*® II RT/RI.

Recommended PCR Reaction Component and conditions (50 µl reaction volumes)

Component	Volume	Final Concentration
cDNA	2 µl	As required
Forward Primer (10 µM)	1 µl	0.2 µM
Reverse Primer (10 µM)	1 µl	0.2 µM
2×*TransTaq*® HiFi PCR SuperMix II	25 µl	1×
Nuclease-free Water	Variable	-

PCR

94℃	2-5 min	
94℃	30 sec	35-40 cycles
50-60℃	30 sec	
72℃	1-2 kb/min	
72℃	5-10 min	

Notes

- Avoid RNase contamination.
- To ensure successful reverse transcription, use high-quality RNA templates.
- For complex RNA templates, or to obtain higher synthesis efficiency, it is recommended to mix RNA template and RNase-free Water well first, incubate at 65°C for 5 minutes, and put on ice for 2 minutes before adding other reaction components.
- Mixing all the reaction components in one step can complete most reverse transcription reactions. For complex RNA templates, or to obtain higher synthesis efficiency, it is recommended to add thermal incubation steps for the template and primers according to the instructions.

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