

TransScript® Two-Step RT-PCR SuperMix

Cat. No. AT401

Storage: at -20°C for two years

Description

TransScript® Two-Step RT-PCR SuperMix performs first-strand cDNA synthesis and PCR in two steps. 5×TransScript® All-in-One SuperMix for PCR is used for reverse transcription and 2×TransTaq® HiFi PCR SuperMix II is used for PCR.

Highlights

Amplification of fragment up to 12 kb.

Application

Multiple copy and low copy gene detection

Kit Contents

Component	AT401-01
5×TransScript® All-in-One SuperMix for PCR	200 µl
2×TransTaq® HiFi PCR SuperMix II	2×1 ml
RNase-free Water	1 ml

Procedures

First-strand cDNA synthesis

1. Reaction Components

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

Optional: for higher efficiency, suggest to mix RNA, primer and water first. Incubate the mixture at 65°C for 5 minutes, on ice for 2 minutes. Then add other components.

2. Incubation

- For RNA template with poly(A)⁺, incubate at 42°C for 30 minutes.
- For RNA template without poly(A)⁺, incubate at 25°C for 10 minutes, then at 42°C for 30 minutes.

3. Incubate at 85°C for 5 seconds to inactivate enzymes.

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μ M)	1 μ l	0.2 μ M
Reverse Primer (10 μ M)	1 μ l	0.2 μ M
2 $\times$ TransTaq [®] HiFi PCR SuperMix II	25 μ l	1 $\times$
Nuclease-free Water	Variable	-
Total volume	50 μ l	-

Thermal cycling conditions

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

FOR RESEARCH USE ONLY