TransScript® II All-in-One First-Strand cDNA Synthesis SuperMix for PCR

Cat. No. AH321

Storage: at -20°C for one year

Description

TransScript® II All-in-One First-Strand cDNA Synthesis SuperMix for PCR provides all the necessary components for cDNA synthesis from total RNA or mRNA. The SuperMix is provided at 5× concentration and used at 1× concentration by adding RNA and H_2O. The resulting cDNA is suitable for regular PCR, not for qPCR.

Highlights

- One-tube format for simple and fast setup and reduced pipetting variability.
- The optimal ratio of oligo$(dT)_{20}$ primer to random primer(N9) for PCR ready cDNA.
- PCR ready cDNA in 30 minutes (unsuitable for qPCR).
- cDNA up to 15 kb.

Applications

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

Kit Contents

Components	AH321-01 (50 rxns)
5×*TransScript*® II All-in-One SuperMix for PCR	200 µl
RNase-free Water	1 ml

Procedures

First-Strand cDNA synthesis

1. Reaction Components

Components	Volume
Total RNA/mRNA	50 ng-5 µg/5-500 ng
5×*TransScript*® II All-in -One SuperMix for PCR	4 µl
RNase-free Water	to 20 µl

Optional: for higher efficiency, suggest to mix RNA 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 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 enzymes.

Reaction Components

Component	Volume	Final Concentration
Template	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×
ddH_2O	Variable	-
Total volume	50 µl	-

Thermal cycling conditions

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

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