TransScript® II First-Strand cDNA Synthesis SuperMix

Cat. No. AH301

Storage: at -20°C for two years

Description

TransScript® II First-Strand cDNA Synthesis SuperMix provides all the necessary components for cDNA synthesis from total RNA or mRNA. The cDNA first strand is efficiently synthesized by *TransScript*® II RT/RI Enzyme Mix and 2×TS II Reaction Mix at 42-55°C, of which the optimum temperature is 50°C.

- Deficient RNase H activity to reduce RNA template degradation during the first-strand cDNA synthesis to ensure its synthesis length and yield..
- The product obtained from 15 minutes reaction is used for qPCR; the product obtained from 30 minutes reaction is used for PCR.
- Anchored Oligo(dT)$_{20}$ Primer is specifically designed to bind to the first base next to the 5' end of Poly(A) tail of mRNA, providing higher specificity and high efficiency for first-strand cDNA synthesis.
- Random Primer (N9) or Gene Specific Primer (GSP) can be used to synthesize the first-strand cDNA.
- 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

Component	AH301-02	AH301-03
TransScript® II RT/RI Enzyme Mix	50 µl	100 µl
2×TS II Reaction Mix	500 µl	1 ml
Random Primer (N9) (0.1 µg/µl)	50 µl	100 µl
Anchored Oligo(dT)$_{20}$ Primer (0.5 µg/µl)	50 µl	100 µl
RNase-free Water	500 µl	1 ml

First-Strand cDNA synthesis

1. Prior to use, please centrifuge all the components briefly.

Component	Volume
Total RNA/mRNA	0.1 ng-5 µg/10 pg-500 ng
Anchored Oligo(dT)$_{20}$ Primer (0.5 µg /µl)	1 µl
or Random Primer(N9) (0.1 µg/µl)	1 µl
or GSP	2 pmol
2×TS II Reaction Mix	10 µl
TransScript® II RT/RI Enzyme Mix	1 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 anchored oligo(dT)$_{20}$ primer or GSP, incubate at 50°C for 15 minutes (for qPCR) or incubate at 50°C for 30 minutes (for PCR).
- For random primer, incubate at 25°C for 10 minutes. After that, incubate at 50°C for 15 minutes (for qPCR) or incubate at 50°C for 30 minutes (for PCR).
- 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.

Recommended Reaction Condition for PCR Amplification.

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

Thermal cycling conditions

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

Recommended Reaction Condition for qPCR.

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	0.4 μl	0.2 μM
Reverse Primer (10 μM)	0.4 μl	0.2 μM
2×*TransStart*® Top/Tip Green qPCR SuperMix	10 μl	1×
Passive Reference Dye (50×) (optional)	0.4 μl	1×
Nuclease-free Water	Variable	-
Total volume	20 μl	-

qPCR（3 steps）

94°C	30 sec	
94°C	5 sec	40-45 cycles
50-60°C	15 sec★	
72°C	10 sec★	

Dissociation Stage

qPCR（2 steps）

94°C	30 sec	
94°C	5 sec	40-45 cycles
60°C	30 sec★	

Dissociation Stage

Fluorescent signals can be collected during the annealing or extension stage. For ABI qPCR instrument, we suggest using the following exposure time:

* For ABI Prism® 7700/7900, set the exposure time to 30 seconds.
* For ABI Prism® 7000/7300, set the exposure time to 31 seconds.
* For ABI Prism® 7500, set the exposure time to 34 seconds.
* For ABI ViiA® 7, set the exposure time at least 19 seconds.

Two-step qPCR is more suitable for higher specificity assay.
Three-step qPCR is more suitable for higher amplification efficiency assay.

Note

- Avoid RNase contamination.
- Use high-quality, intact RNA for accurate qualification in RT-PCR.
- For complex template or higher synthesis efficiency, it is suggested to add heat incubation step for template and primers according to the manual, though most reverse transcription reaction can be finished successfully by mixing all the reaction components at one-step.
- When the product is used for qPCR, it is suggested to extend the incubation time at 50°C to 30 minutes to achieve better amplification result for some particular genes.

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