TransScript® II One-Step gDNA Removal and cDNA Synthesis SuperMix

Cat. No. AH311
Storage: at -20°C for two years

Descriptions

Unique genomic DNA remover is combined with *TransScript*® II First- Strand cDNA Synthesis SuperMix to achieve simultaneous genomic DNA removal and cDNA synthesis at 42-55°C. After cDNA synthesis, gDNA remover and *TransScript*® II RT/RI Enzyme Mix are inactivated by heating at 85°C for 5 seconds.

Highlights

- Simultaneous genomic DNA removal and cDNA synthesis in one tube to minimize RNA contamination.
- The product obtained from 15 minutes reaction is used for qPCR; the product obtained from 30 minutes reaction is used for PCR.
- After reaction, heat inactivation for RT/RI and gDNA remover simultaneously can avoid damage to RNA caused by heat inactivating DNase I used for pretreatment in traditional protocol.
- Simple and fast procedure.
- 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	AH311-02 (50 rxns)	AH311-03 (100 rxns)
TransScript® II RT/RI Enzyme Mix	50 µl	100 µl
gDNA Remover	50 µl	100 µl
2×TS II Reaction Mix	500 µl	1 ml
Random Primer (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

Procedures

First-strand cDNA synthesis

1. Mix all the components.

Components	Volume
Total RNA/mRNA	50 ng-5 µg/5-500 ng
Anchored Oligo(dT)$_{20}$ Primer (0.5 µg/µl)	1 µl
or Random Primer (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
gDNA Remover	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 Reacton Condition for PCR Amplification.

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	-
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	5-10 min	

Recommended Reacton 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

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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