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. After cDNA synthesis, gDNA remover and reverse transcriptase 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.
- 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. Reaction Component

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.

- 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.

Reaction Components

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	

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