

TransScript® One-Step gDNA Removal and cDNA Synthesis SuperMix

Cat. No. AT311

Storage: at -20°C for two years

Description

Unique genomic DNA remover is combined with *TransScript*® 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 12 kb.

Applications

Multiple copy and low copy gene detection

Kit Contents

Component	AT311-02 (50 rxns)	AT311-03 (100 rxns)	AT311-04 (500 rxns)
TransScript® RT/RI Enzyme Mix	50 μl	100 μl	5×100 μ1
gDNA Remover	50 μl	100 μl	5×100 μl
2×TS Reaction Mix	500 μl	1 ml	5×1 ml
Random Primer(N9) (0.1 µg/µl)	50 μl	100 μl	5×100 μl
Anchored Oligo(dT) ₁₈ Primer (0.5 μg/μl)	50 μl	100 μl	5×100 μl
RNase-free Water	500 μl	1 ml	5 ml

First-Strand cDNA synthesis

1. Reaction Components

Component	Volume
Total RNA/mRNA	0.1 ng-5 μg/10 pg-500 ng
Anchored Oligo(dT) ₁₈ Primer (0.5 μg/μl)	1 μ1
or Random Primer (0.1 μg/μl)	1 μ1
or GSP	2 pmol
2×TS Reaction Mix	10 μ1
TransScript® RT/RI Enzyme Mix	1 μ1
gDNA Remover	1 μ1
RNase-free Water	to 20 μl

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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)₁₈ primer or GSP, incubate at 42°C for 15 minutes (for qPCR) or incubate at 42°C for 30 minutes (for PCR).
- For random primer, incubate at 25°C for 10 minutes. After that, incubate at 42°C for 15 minutes (for qPCR) or incubate at 42°C for 30 minutes (for PCR).
- 3. Incubate at 85°C for 5 seconds to inactivate enzymes.

RT-PCR

Reaction Components

Component	Volume	Final Concentration
cDNA	2 μ1	as required
Forward Primer (10 µM)	1 μl	0.2 μΜ
Reverse Primer (10 μM)	1 μl	0.2 μΜ
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 —	
50-60°C	30 sec	30-35 cycles
72°C	1-2 kb/min	J
72°C	5-10 min	