

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

Cat. No. AU311

Storage: at -20°C for two years

Description

TransScript®-Uni RT is an improved version of M-MLV reverse transcriptase with broad range of reaction temperature (42°C-65°C) and higher thermostability. The suggested reaction temperature is 50°C. The SuperMix contains reagents for 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

- Broad range reaction temperature (42°C-65°C) .
- 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 20 kb.

Applications

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

Kit Contents

Component	AU311-02	AU311-03
TransScript®-Uni RT/RI Enzyme Mix	50 µl	100 µl
gDNA Remover	50 µl	100 µl
2×TS-Uni 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 and gDNA removal

1. Reaction Components

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 (0.1 µg/µl)	1 µl
or GSP	2 pmol
2×TS-Uni Reaction Mix	10 µl
TransScript®-Uni 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, 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, at incubate 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, better yield can be obtained by optimizing the reaction temperature.

3. Incubate at 85°C for 5 seconds to inactivate enzymes.

RT-PCR

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× <i>TransTaq</i> [®] HiFi PCR SuperMix II	25 µl	1×
Nuclease-free Water	Variable	-
Total volume	50 µl	-

Thermal cycling conditions

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

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