

TransScript® One-Step gDNA Removal and cDNA Synthesis SuperMix

Please read the manual carefully before use.

Cat. No. AT311

Version No. Version 2.0

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, *TransScript*® RT/RI and gDNA remover are inactivated by heating at 85°C for 5 seconds.

Features

- 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.
- Easy to operate.
- 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)
<i>TransScript</i> ® RT/RI Enzyme Mix	50 µl	100 µl	5×100 µl
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

Briefly spin each component before use.

First-Strand cDNA synthesis and Genomic DNA Removal

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 µl
or Random Primer (N9) (0.1 µg/µl)	1 µl
or GSP	2 pmol
2×TS Reaction Mix	10 µl
<i>TransScript</i> ® RT/RI Enzyme Mix	1 µl
gDNA Remover	1 µl
RNase-free Water	Variable
Total volume	20 µl



2. Mix gently

- 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 *TransScript*[®] RT/RI and gDNA Remover.

Recommended qPCR system and conditions (taking 20 µl reaction system as an example)

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× <i>PerfectStart</i> [®] Green qPCR SuperMix	10 µl	1×
Passive Reference Dye (50×) (optional)	0.4 µl	1×
Nuclease-free Water	Variable	-
Total volume	20 µl	-

qPCR (three-step)

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

40-45 cycles

Dissociation Stage

For ABI instruments, fluorescent signals can be collected during the annealing or extension stage in the three-step method, and read time is as follows:

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

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

Recommended PCR system and conditions (taking 50 µl reaction system as an example)

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

PCR

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

35-40 cycles



Notes

- Avoid RNase contamination.
- To ensure successful reverse transcription, use high-quality RNA templates.
- For complex RNA templates, or to obtain higher synthesis efficiency, it is recommended to mix RNA template and RNase-free Water well first, incubate at 65°C for 5 minutes, and put on ice for 2 minutes before adding other reaction components.
- Mixing all the reaction components in one step can complete most reverse transcription reactions. For complex RNA templates, or to obtain higher synthesis efficiency, it is recommended to add thermal incubation steps for the template and primers according to the instructions.
- If the product is used for qPCR, for some special genes, the incubation time at 42 °C can be 30 minutes to obtain better results.





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