TransScript® Uni All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal)

Please read the mannual carefully before use.

Cat. No. AU341

Version No. Version 3.0

Storage: at -18°C or below for two years.

Description

The kit provides all the necessary reagents for reverse transcription reaction (including *TransScript*® Uni RT, RNase Inhibitor, Anchored Oligo $(dT)_{20}$ Primer, Random Primer (N9), dNTPs, Buffer). During the reaction, simply add the 5× All-in-One Reaction Mix for qPCR, *TransScript*® Uni All-in-One Enzyme Mix, RNA template, and H_2O. Under conditions ranging from 42°C to 65°C, the system enables efficient first-strand cDNA synthesis while simultaneously removing residual genomic DNA from the RNA template. 20×No RT Control Mix is supplied to prepare no-reverse transcriptase (RT) control, which is used to assess if the qPCR template is contaminated with genomic DNA. This product is capable of minimizing contamination during operation with a simple workflow.

The resulting cDNA is only suitable for qPCR, not for regular PCR.

Highlights

- Board range reaction temperature (42°C-65°C).
- All SuperMix form: Simultaneous cDNA synthesis and genomic DNA removal by only adding the 5× All-in-One Reaction Mix for qPCR, *TransScript*® UniAll-in-One Enzyme Mix, RNA template, and H_2O.
- High synthesis efficiency enabled by optimal ratio of $Oligo(dT)_{20}$ Primer to Random Primer (N9) and optimized composition of the SuperMix, ensuring same reverse transcription efficiency for RNA templates of different concentrations and specifically high synthesis efficiency for short cDNA.
- Only 5 minutes for reverse transcription.
- High compatibility with qPCR reagents.

Applications :High-copy number and low-copy number gene detection

Kit Contents

Component	AU341-02 (100 rxns)
5×All-in-One Reaction Mix for qPCR	400 µl
TransScript® Uni All-in-One Enzyme Mix	100 µl
20×No RT Control Mix	40 µl
RNase-free Water	2×1 ml

Prior to use, please centrifuge each component briefly.

First-strand cDNA synthesis and gDNA removal

1. Reverse transcription reaction mix and no-RT control reaction mix (optional)

Component	Volume (RT Reaction)	Volume (No RT Control)
Total RNA/mRNA	≤1 µg/ ≤100 ng	≤1 µg/ ≤100 ng
5×All-in-One Reaction Mix for qPCR	4 µl	4 µl
TransScript® Uni All-in-One Enzyme Mix	1 µl	-
20×No RT Control Mix	-	1 µl
RNase-free Water	Variable	Variable
Total volume	20 µl	20 µl

2. Mix well gently, and incubate at 50°C for 5 minutes
   For GC-rich or complex secondary structure RNA template, it is suggested to increase the reaction temperature to some extent(≤65°C).
3. Incubate at 85°C for 2 minutes to inactivate *TransScript*® Uni 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×*PerfectStart*® Green qPCR SuperMix	10 μl	1×
Universal Passive Reference Dye (50×) (optional)	0.4 μl	1×
Nuclease-free Water	Variable	-
Total volume	20 μl	-

qPCR (Three-step)

94℃ 30 sec
94℃ 5 sec
50-60℃ 15 sec★
72℃ 10 sec★
40-45 cycles
Dissociation stage

qPCR (Two-step)

94℃ 30 sec
94℃ 5 sec
60℃ 30 sec★
40-45 cycles
Dissociation stage

For ABI qPCR instrument, we suggest using the following read time (Fluorescent signals can be collected during the annealing or extension stage for three-step qPCR):

★ For ABI Prism7700/7900, set the read time to 30 seconds.
★ For ABI Prism7000/7300, set the read time to 31 seconds.
★ For ABI Prism7500, set the read time to 34 seconds.
★ For ABI ViiA7, set the read time is 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.

Universal Passive Reference Dye Compatibility with Different Instruments

• Universal Passive Reference Dye (50×)

ABI Prism 7000/7300/7700/7900, ABI Step One, ABI Step One Plus, ABI 7900HT, ABI 7900HT Fast, ABI Prism 7500, ABI Prism 7500 Fast, ABI QuantStudio Dx/3/5, ABI QuantStudio 6/7/12K Flex, ABI ViiA 7, Stratagene Mx3000P/Mx3005P/Mx4000

• No Passive Reference Dye

Roche LightCycler 480, Roche Light Cycler 96, MJ Research Chromo4, MJ Research Opticon 2, Takara TP-800, Bio-Rad iCycler iQ, Bio-Rad iCycler iQ5, Bio-Rad CFX96, Bio-Rad C1000 Thermal Cycler, Thermo Scientific Pikoreal 96, Qiagen Corbett Rotor-Gene 6000, Qiagen Corbett Rotor-Gene G, Qiagen Corbett Rotor-Gene Q, Qiagen Corbett Rotor-Gene 3000, Mastercycler ep realplex

Notes

• For complex RNA template, or to obtain higher synthesis efficiency, it is recommended to mix RNA template and RNase-free Water well, incubate at 65°C for 5 minutes, and put on ice for 2 minutes before adding other reaction components.
• Avoid RNase contamination.
• Use high-quality, intact RNA templates to ensure the success of reverse transcription.

For research use only, not for clinical diagnosis.

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