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

Please read the manual carefully before use.

Cat. No. AT342

Version No. Version 1.0

Storage: at -18˚C or below for two years

Description

This product contains all reagents required for the reverse transcription reaction (*TransScript*® RT, RNase Inhibitor, Anchored Oligo(dT)$_{18}$ Primer, Random Primer(N9), dNTPs, Buffer), at a concentration of 5×. During the reaction, simply add gDNA Remover, template RNA, and water to efficiently synthesize first-strand cDNA, while simultaneously removing residual genomic DNA from the RNA template. A 5×*TransScript*® Visual All-in-One No-RT Control SuperMix for qPCR is also provided, used to prepare a no-reverse-transcriptase control to determine whether the qPCR template is derived from cDNA. This product is easy to use and reduces the chance of contamination during operation.

The product contains a blue tracking dye, enabling visual pipetting. It can be used in combination with a universal dye-based fluorescence quantitative PCR premix (Catalog No.: AQ641) to achieve visual operation throughout the entire reverse transcription and qPCR amplification process. cDNA is only suitable for qPCR, not for conventional PCR.

Features

- All SuperMix format: Simply add gDNA Remover, template RNA, and water to achieve cDNA synthesis and genomic DNA removal simultaneously.
- Optimal ratio of Oligo(dT)$_{18}$ Primer and Random Primer(N9), combined with an optimized SuperMix composition, ensures consistent reverse transcription efficiency for different concentrations of RNA, with high short-strand cDNA synthesis efficiency.
- Reverse transcription takes only 15 minutes.
- High compatibility with qPCR reagents.
- Contains a blue tracking dye for visual pipetting.

Applications

Detection of high-copy and low-copy genes.

Kit Contents

Component	AT342-01 (50 rxns)	AT342-02 (100 rxns)	AT342-03 (500 rxns)
5×*TransScript*® Visual All-in-One SuperMix for qPCR	200 µl	400 µl	5×400 µl
5×*TransScript*® Visual All-in-One No-RT Control SuperMix for qPCR	20 µl	40 µl	200 µl
gDNA Remover	50 µl	100 µl	5×100 µl
RNase-free Water	1 ml	2×1 ml	2×5 ml
1×cDNA Dilution Buffer	1 ml	2×1 ml	2×5 ml

Before use, please briefly spin down each component.

First-strand cDNA synthesis and gDNA removal

1. Reverse transcription reaction system and No RT Control reaction system (optional)

Component	Volume(RT reaction)	Volume(No RT Control)
Total RNA/mRNA	≤1 μg/ ≤100 ng	≤1 μg/ ≤100 ng
5×*TransScript*® Visual All-in-One SuperMix for qPCR	4 μl	-
5×*TransScript*® Visual All-in-One No-RT Control SuperMix for qPCR	-	4 μl
gDNA Remover	1 μl	1 μl
RNase-free Water	Variable	Variable
Total volume	20 μl	20 μl

2. Mix gently and incubate at 42°C for 15 minutes.

3. Heat at 85°C for 2 minutes to inactivate *TransScript*® RT/RI and gDNA Remover.

Recommended qPCR system and conditions (using a 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*® IV Visual Green qPCR SuperMix	10 μl	1×
Nuclease-free Water	Variable	-
Total volume	20 μl	-

qPCR (two-step method)

95℃ 1 min

95℃ 5 sec

60℃ 30 sec★ ⌝ 40-45 cycles

Dissociation stage

For ABI instruments, the time for the fluorescence signal acquisition step (which can be the annealing or extension step in the three-step method) is as follows:

★ When using ABI Prism 7700/7900, set the acquisition time to 30 seconds;

★ When using ABI Prism 7000/7300, set the acquisition time to 31 seconds;

★ When using ABI Prism 7500, set the acquisition time to 34 seconds;

★ When using ABI ViiA 7, set the acquisition time to at least 19 seconds.

Passive Reference Dye compatible instrument models

• Passive Reference Dye I (50×)

ABI Prism 7000/7300/7700/7900, ABI Step One, ABI Step One Plus, ABI 7900HT, ABI 7900HT Fast

• Passive Reference Dye II (50×)

ABI Prism 7500, ABI Prism 7500 Fast, ABI QuantStudio 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 Q, Qiagen Corbett Rotor-Gene 3000, Mastercycler ep realplex

Notes

- For complex RNA templates, or to obtain higher synthesis efficiency, it is recommended to mix the RNA template with RNase-free Water, incubate at 65°C for 5 minutes, then place on ice for 2 minutes, and then add the other reaction components.
- Avoid RNase contamination.
- To ensure successful reverse transcription, please use a high-quality RNA template.
- 1× cDNA Dilution Buffer can be used to dilute cDNA.

For research use only, not for clinical diagnosis.

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