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

Cat. No. AE341
Storage: at -20°C for two years

Description

5×*EasyScript*® All-in-One SuperMix for qPCR provides all the necessary reagents for cDNA synthesis from total RNA or mRNA (including 5×*EasyScript*® RT, RNase Inhibitor, Anchored Oligo $(dT)_{18}$ Primer, Random Primer (N9), dNTPs, Buffer). It is provided at 5× concentration and used at 1× concentration by only adding gDNA remover, RNA template and H_2O for efficient first-strand cDNA synthesis. Simultaneously, residual genomic DNA from RNA template can be removed. 5×*EasyScript*® All-in-One No-RT Control SuperMix for qPCR 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.

Hightlights

• "All-in-One SuperMix" type: Simultaneous cDNA synthesis and genomic DNA removal by only adding gDNA remover, RNA template and H_2O
• High synthesis efficiency enabled by optimal ratio of Oligo$(dT)_{18}$ 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 15 minutes for reverse transcription.
• High compatibility with qPCR reagents.

Applications

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

Kit Contents

Component	AE341-02 (100 rxns)	AE341-03 (500 rxns)
5×*EasyScript*® All-in-One SuperMix for qPCR	400 µl	5×400 µl
5×*EasyScript*® All-in-One No-RT Control SuperMix for qPCR	40 µl	200 µl
gDNA Remover	100 µl	5×100 µl
RNase-free Water	2×1 ml	2×5 ml

Prior to use, please centrifuge each component briefly.

First-strand cDNA synthesis and gDNA removal

1. Add reaction components according to the following table:

Component	Volume
Total RNA/mRNA	≤1µg / ≤100 ng
5×*EasyScript*® All-in-One SuperMix for qPCR	4 µl
gDNA Remover	1 µl
RNase-free Water	Variable
Total volume	20 µl

2. Mix well gently, and incubate at 42°C for 15 minutes.
3. Incubate at 85°C for 5 seconds to inactivate enzymes and gDNA Remover.

Recommended qPCR Components and Conditions (20 µl)

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	1x
Passive Reference Dye (50×) (optional)	0.4 µl	1x
Nuclease-free Water	Variable	-
Total Volume	20 µl	-

qPCR (three-step)

94°C	30 sec	
94°C	5 sec	40-45 cycles
50-60°C	15 sec*	
72°C	10 sec*	

qPCR (two-step)

94°C	30 sec	
94°C	5 sec*	40-45 cycles
60°C	30 sec*	

Dissociation Stage

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

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

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

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

Passive Reference Dye Compatibility with Different Instruments

• 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 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.

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