EasyScript® First-Strand cDNA Synthesis SuperMix

Cat. No. AE301

Storage: at -20°C for two years

Description

EasyScript® First-Strand cDNA Synthesis SuperMix provides all the necessary components for cDNA synthesis from total RNA or mRNA. The cDNA is efficiently synthesized by *EasyScript*® RT/RI Enzyme Mix and 2×ES Reaction Mix.

- Deficient RNase H activity to reduce RNA template degradation during the first-strand cDNA synthesis.
- The product obtained from 15 minutes reaction is used for qPCR; the product obtained from 30 minutes reaction is used for PCR.
- Anchored Oligo$(dT)_{18}$ Primer is specifically designed to bind to the first base next to mRNA Poly$(A)^+$ on the 5' end with high specificity, ensuring high efficiency and success rate of first-strand cDNA synthesis.
- Random Primer (N9) or Gene Specific Primer (GSP) can be used to synthesize the first-strand cDNA.
- cDNA up to 8 kb.

Application

Multiple copy gene detection

Kit Contents

Component	AE301-02	AE301-03
EasyScript® RT/RI Enzyme Mix	50 µl	100 µl
2×ES Reaction Mix	500 µl	1 ml
Random Primer(N9) (0.1 µg/µl)	50 µl	100 µl
Anchored Oligo$(dT)_{18}$ Primer (0.5 µg/µl)	50 µl	100 µl
RNase-free Water	500 µl	1 ml

First-Strand cDNA synthesis

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×ES Reaction Mix	10 µl
EasyScript® RT/RI Enzyme Mix	1 µl
RNase-free Water	to 20 µl

Optional: For complex RNA templates, to obtain higher synthesis efficiency, it is recommended to mix RNA template, primer and RNase-free water well, incubate the mixture at 65°C for 5 minutes and on ice for 2 minutes, and then add other components.

2. Incubation

- 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 enzymes.

RT-PCR

Recommended Reaction Condition for qPCR (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×*TransStart*® Top/Tip Green qPCR SuperMix	10 μl	1×
Passive Reference Dye (50×) (optional)	0.4 μl	1×
Nuclease-free Water	Variable	-
Total volume	20 μl	-

qPCR（3 steps）

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

Dissociation Stage

qPCR（2 steps）

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

Dissociation Stage

For ABI qPCR instrument, we suggest using the following signal collecting time (annealing or extension stage for three-step method):

★For ABI Prism7700/7900, the time is 30 seconds. ★For ABI Prism7000/7300, the time is 31 seconds.

★ For ABI Prism7500, the time is 34 seconds. ★For ABI ViiA 7, the time is at least 19 seconds.

Three-step qPCR is suitable to get higher amplification efficiency.

Two-step qPCR is suitable to get higher specificity.

Recommended Reaction Condition for PCR (50 μl)

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×*TransTaq*® HiFi PCR SuperMix II	25 μl	1×
Nuclease-free Water	Variable	-
Total volume	50 μl	-

PCR

94℃	2-5 min	
94℃	30 sec	35-40 cycles
50-60℃	30 sec	
72℃	1-2 kb/min	
72℃	5-10 min	

Note

- Avoid RNase contamination.
- High-quality RNA template should be used to ensure the success of reverse transcription.
- Most reverse transcription reactions can be finished successfully by mixing all the reaction components at one step. For complex RNA template, to get higher synthesis efficiency, it is suggested to add heat incubation steps of template and primers according to the manual.
- When the product is used for qPCR, it is suggested to extend the incubation time at 42°C to 30 minutes to achieve better amplification results for some particular genes.

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