EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix

Please read the manual carefully before use

Cat. No. AE311

Version No. Version 2.1

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

Description

Unique genomic DNA remover is combined with *EasyScript*® First- Strand cDNA Synthesis SuperMix to achieve simultaneous genomic DNA removal and cDNA synthesis. After cDNA synthesis, *EasyScript*® RT/RI and gDNA remover are inactivated by heating at 85°C for 2 minutes.

Features

• Simultaneous genomic DNA removal and cDNA synthesis in one tube, streamlining the workflow and minimizing 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 8 kb.

Applications

High-copy gene detection

Kit Contents

Component	AE311-02 (50 rxns)	AE311-03 (100 rxns)	AE311-04 (500 rxns)
EasyScript® RT/RI Enzyme Mix	50 μl	100 μl	5×100 μl
gDNA Remover	50 μl	100 μl	5×100 μl
2×ES Reaction Mix	500 μl	1 ml	5×1 ml
Random Primer (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×ES Reaction Mix	10 μl
EasyScript® 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 (N9), 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 2 minutes to inactivate *EasyScript*® 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×
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★ ⎥ 40-45 cycles
72℃ 10 sec★ ⎦
Dissociation Stage

qPCR (two-step)

94℃ 30 sec
94℃ 5 sec ⎤ 40-45 cycles
60℃ 30 sec★ ⎦
Dissociation Stage

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

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

PCR

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

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, primers, 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 step for the template and primers according to the instruction.
• 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.

For research use only, not for clinical diagnosis.

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