EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix

Cat. No. AE311

Storage: at -20°C 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, gDNA remover and reverse transcriptase are inactivated by heating at 85°C for 5 seconds.

Hightlights

- Simultaneous genomic DNA removal and cDNA synthesis in one tube to minimize RNA contamination.
- The product obtained from 15 minutes reaction is used for qPCR; the product obtained from 30 minutes reaction is used for PCR.
- cDNA up to 8 kb.

Applications

Multiple copy gene detection

Kit Contents

Component	AE311-02 (50 rxns)	AE311-03 (100 rxns)
EasyScript® RT/RI Enzyme Mix	50 µl	100 µl
gDNA Remover	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	50 ng -5 µg/5-500 ng
Anchored Oligo(dT)$_{18}$ Primer (0.5 µg/µl)	1 µl
or Random Primer (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	to 20 µl

Optional: for higher efficiency, suggest to mix RNA, primer and water first. Incubate the mixture at 65°C for 5 minutes, on ice for 2 minutes. 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

Reaction Components

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
10×*TransTaq*® HiFi Buffer II	5 μl	1×
2.5 mM dNTPs	4 μl	0.2 mM
TransTaq® HiFi DNA Polymerase	0.5-1 μl	2.5-5 units
ddH_2O	Variable	-
Total volume	50 μl	-

Thermal cycling conditions

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

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