

EasyScript® First-Strand cDNA Synthesis SuperMix

Cat. No. AE301

Storage: at -20°C for one year

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 for higher yield and more full length 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	50 ng -5 µg/5-500 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 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
2× <i>TransTaq</i> [®] HiFi PCR SuperMix II	25 µl	1×
ddH ₂ O	Variable	-
Total volume	50 µl	-

Thermal cycling conditions

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

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