

EasyScript[®] Reverse Transcriptase [M-MLV, RNase H⁻]

Cat. No. AE101

Storage: at -20°C for one year

Concentration: 200 units/μl

Description

EasyScript[®] Reverse Transcriptase is an engineered version of M-MLV reverse transcriptase with deficient RNase H activity. The enzyme is purified to near homogeneity from E.coli containing the modified M-MLV RT gene.

- Deficient RNase H activity to reduce RNA template degradation during the first-strand cDNA synthesis.
- Anchored Oligo(dT)₁₈ Primer for higher yield and more full length cDNA.
- cDNA up to 8 kb.

Applications

- First-strand cDNA synthesis
- Multiple copy gene detection

Unit Definition

One unit of EasyScript[®] RT incorporates 1 nmol of deoxyribonucleotide into acid precipitable material in 10 minutes at 37°C using Poly(A)/Oligo(dT) as template/primer.

5×ES RT Buffer

375 mM KCl, 15 mM MgCl₂, 100 mM Tris-HCl pH 8.4

Kit Contents

Component	AE101-02	AE101-03
EasyScript [®] RT	10000 U	5×10000 U
5×ES RT Buffer	200 μl	5×200 μl
Anchored Oligo(dT) ₁₈ Primer (0.5 μg/μl)	50 μl	250 μl

First-Strand cDNA synthesis reactions

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
10 mM dNTPs	1 μl
5×ES RT Buffer	4 μl
Ribonuclease Inhibitor (50 units/μl)	0.5 μl
EasyScript [®] RT	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 30 minutes.
 - For random primer, incubate at 25°C for 10 minutes, then at 42°C for 30 minutes.
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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