AddScript Reverse Transcriptase

Research Use Only

Product Code

21001

Component

- 1. 20x AddScript Reverse Transcriptase 50 µl
- 2. 2x Reaction Buffer 0.5 ml

Storage Condition

Store at -20°C

Description

AddScript Reverse Transcriptase is a mutant of MMLV Reverse Transcriptase with reduced RNase H activity and increased thermal stability.

Usage Information

- The reaction temperature for cDNA synthesis is 50℃.
- The reaction time for cDNA synthesis is 60 min.

Quality Control

The performance of AddScript Reverse Transcriptase is tested in an RT reaction using human total RNA with oligo dT_{20} and random hexamer each. The sensitivity of the kit is verified by the detection of GAPDH and Actin transcript in 10 pg total RNA after 30 cycles.

Storage and Stability

AddScript Reverse Transcriptase is stable for 2 years when stored in a constant temperature freezer at less than -20°C.

Reaction Assembly

1. Add the following components to a thin-walled PCR tube:

Nuclease-free D.W	x μl
2x Reaction Buffer	10 μΙ
10mM dNTP Mixture (Not provided)	2.0 μΙ
50~100 pmoles/µl oligo dT ₂₀ (random hexamer) or Gene specific primer (10~20 pmoles/µl)	1.0 μΙ
RNA template	x μl
RNase Inhibitor (Optional)	x μl
20x AddScript Reverse Transcriptase	1.0 μΙ
Total reaction volume	20 μΙ

^{*} Recommendation for template RNA concentration in a 20 μ l reaction volume

2. Temperature cycling Protocol

Priming	25℃, 10 min
Reverse transcription	50°C, 60 min
RT inactivation	80°C, 5 min
Hold	12℃, ∞

Recommendation for downstream PCR

For downstream PCR amplification, the volume of cDNA product should not exceed 1/5 of the PCR reaction volume, typically 1 \sim 4 μ l in 20 μ l PCR reaction.

¹⁾ total RNA: 100 fg \sim 1 μg

²⁾ mRNA: 10 fg \sim 1 μg