Cat.: E42

Neoscript Reverse Transcriptase-V2 V01

Product Description

Neoscript Reverse Transcriptase-V2 is a high-activity reverse transcriptase developed through rational screening platform-based directed evolution based on Biori Neoscript RTase. It features enhanced activity, improved temperature tolerance, and superior inhibitor tolerance, making it suitable for high-temperature reverse transcription and rapid reverse transcription. This enzyme effectively eliminates the adverse effects of RNA secondary structure and non-specific factors on cDNA synthesis, offering superior stability and reverse transcription synthesis capability.

Components

1. 200 U/μL Neoscript Reverse Transcriptase-V2
2. 5×First-Strand Buffer (optional)

*5×First-Strand Buffer does not contain dNTPs or Mg^{2+}. Please add dNTPs and $MgCl_2$ when preparing reaction systems.

Unit Definition

One unit of activity (U) refers to the amount of enzymes required to incorporate 1 nmol dTTP into poly(A)•Oligo$(dT)_{25}$ template/primer at 37°C within 10 minutes.

Storage

Store at -20±5℃. Vortex before use. Avoid freeze-thaw cycles.

Quality Control

1. SDS-PAGE electrophoresis purity no less than 98%.
2. Amplification sensitivity, batch-batch difference, and stability.
3. No exogenous nuclease activity, no exogenous endonuclease or exonuclease contamination.

First-strand synthesis

1. Prepare the reaction system according to the table below.

Component	Concentration in Master Mix
Oligo $(dT)_{12-18}$ Primer	50 pmol
Or Random Primer *	50 pmol (20-100 pmol)
Or Gene specific Primer *	2 pmol
dNTPs (10 mM each)	1 μL
Template RNA	total RNA≤5 μg, mRNA≤1 μg
RNase-free ddH_2O	To 10 μL

*Select different types of primers according to experimental needs.

2. Heat at 65℃ for 5 minutes, then quickly cool on ice for 2 minutes.
3. Add the following components to the above system until the total volume reaches 20 μL, and gently mix.

Component	Concentration in Master Mix
5×First-Strand Buffer	4 μL
200 U/μL Neoscript Reverse Transcriptase-V2	1 μL
40 U/μL RNase Inhibitor	1 μL
RNase-free ddH_2O	To 20 μL

4. Perform reactions under the following conditions:
   (1) If using Random Primer random primers, incubate at 25℃ for 10 minutes and then at 50℃ for 30-60 minutes.
   (2) If using Oligo dT or specific primers, incubate at 50℃ for 30-60 minutes.
5. Heat inactivate Neoscript RTase Ⅱ by incubating at 95℃ for five minutes and terminate the reaction.
6. The reverse transcription product can be directly used in PCR reactions and fluorescence quantitative PCR reactions or stored long-term at -20℃.

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Prepare Reaction Mix

Components	Volume per Reaction	Final Concentration
10×PCR Buffer Ⅱ (Mg^{2+} free) 1	5 μL	1×
dNTPs (10 mM each)	1 μL	200 μM
25 mM $MgCl_2$	2-8 μL	1-4 mM
5 U/μL Taq DNA Polymersase	0.25-0.5 μL	1.25-2.5U/Reaction
25×Primer Mix [2]	2 μL	1×
Template	——	＜1 μg/Reaction
ddH_2O	To 50 μL	——

1. 10×PCR Buffer Ⅱ (Mg^{2+} free) does not contain dNTP or Mg^{2+}. Please add dNTPs and $MgCl_2$ when preparing reaction system.

2. If used for qPCR/qRT-PCR, fluorescent probes need to be added to the reaction system. The final concentration of primers is usually 0.2 μM for better results. when the reaction performance is poor, the primer concentration can be adjusted within the range of 0.2-1 μM. The probe concentration is usually optimized in the range of 0.1-0.3 μM. Concentration gradient experiments can be performed to find the best combination of primers and probes.

Reaction Programe

Standard PCR Program			
Procedure	Temp	Time	Cycle
Hot Start	95°C	2-5 min	1
Denaturation	95°C	10-20 s	30-40
Annealing	50-60°C	10-30 s	
Extension	72°C	10-60 s	

Notes

1. Suitable for temperature optimization of reverse transcription between 42℃-55℃.
2. Has better stability, suitable for high-temperature reverse transcription amplification, effectively passing through RNA complex structure regions, applicable to one-step multiple fluorescent quantitative RT-PCR detection.
3. Compatible with various PCR amplification enzymes and suitable for highly sensitive RT-PCR reactions.
4. Suitable for highly sensitive one-step fluorescent quantitative RT-PCR reaction, effectively improving the detection rate of low-concentration templates.
5. Suitable for cDNA library construction.
6. 3' and 5'RACE.

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