Cat.:E42-NA

Neoscript Reverse Transcriptase-Low DNA-V2

V01

Product Description

Neoscript Reverse Transcriptase-Low DNA-V2 represents a high - activity reverse transcriptase acquired via the targeted modification of Neoscript RTase utilizing a rational screening platform. This enzyme showcases heightened thermal stability. It effectively alleviating the detrimental impacts of RNA higher - order structures and non - specific factors on cDNA synthesis, thereby manifesting superior stability and reverse transcription synthesis capacity.

Size

200 U/μL

Components

1. 200 U/μL Neoscript RTase Ⅱ (NA)
2. 5×First-Strand Buffer (optional)

*5×First-Strand Buffer does not contain dNTPs. Please add dNTPs when preparing reaction systems.

Unit Definition

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

Storage Buffer

20 mM Tris-HCl (pH7.5), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, Stabilizer.

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, between-batch difference, and stability.
3. No exogenous nuclease activity, no exogenous endo/exonuclease contamination.

Synthesis of the first strand

1. Refer to the following table for system configuration.

Components	Final Conc.
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.
ddH_2O	To 10 μL

2. The reagents were subjected to heating at 65 °C for a duration of 5 minutes, and subsequently followed by rapid cooling on ice for 2 minutes.
3. Incorporate the following components into the previously mentioned system, adjust the total volume to 20 μL, and gently homogenize.

Components	Final Conc.
5×First-Strand Buffer	4 μL
200 U/μL Neoscript RTase Ⅱ (NA)	1 μL
40 U/μL RNase Inhibitor	1 μL
RNase-free ddH_2O	To 20 μL

4. Conduct the reaction under the following conditions.

(1) When employing Random Primer, it should first undergo incubation at 25°C for 10 minutes, followed by incubation at 50°C for 30 - 60 minutes.
(2) When using Oligo dT or specific primers, incubation at 50°C for 30 - 60 minutes is necessary.
5. Incubate at 95°C for 5 minutes to inactivate Neoscript RTase II (NA) and terminate the reaction.
6. The products of reverse transcription can be directly applied to polymerase chain reaction (PCR) and real - time quantitative PCR (qPCR), or stored for an extended duration at -20°C.

Prepare Reaction Mix For PCR

Components	Final Concentration
10×PCR Buffer (Mg^{2+} free)	1×
dNTPs (10 mM each)	200 μM
25 mM $MgCl_2$	1 - 4 mM
5 U/μL Taq DNA Polymersase (NA)	2 - 2.5 U
Primer 1 (10 μM)	0.2 - 1 μM
Primer 2 (10 μM)	0.2 - 1 μM
Template RNA*	≤ 10% the first strand reaction solution (2 μL)
ddH_2O	To 50 μL

*The addition of an excessive amount of the first - strand reaction solution may potentially hinder the PCR reaction.

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

This reagent is suitable for optimizing the reverse transcription temperature within the range of 50°C to 55°C. It exhibits excellent stability, is suitable for high-temperature reverse transcription amplification, can efficiently traverse the complex structural regions of RNA, and is appropriate for one-step multiplex fluorescent quantitative RT-PCR detection. It shows remarkable compatibility with various PCR amplification enzymes and is suitable for high-sensitivity RT-PCR reactions. This reagent is applicable to high-sensitivity one-step fluorescent quantitative RT-PCR reactions, which can effectively improve the detection rate of low-concentration templates. It is suitable for the construction of cDNA libraries and applicable to 3' and 5' RACE.