Cat.: FE43-NA

Neoscript Hot Start Reverse Transcriptase-Low DNA (Glycerol-Free)-V2 V01

Product Description

Neoscript Hot Start Reverse Transcriptase-Low DNA (Glycerol-Free)-V2 represents a high - activity reverse transcriptase acquired via the targeted modification of Neoscript RTase (DG) utilizing a rational screening platform. It is an enzyme that has been specifically optimized and formulated to fulfill the distinctive requirements of lyophilized reagents. This enzyme eradicates RNase H activity and showcases heightened thermal stability. The product undergoes thermal activation modification to suppress reverse transcriptase activity at ambient temperature, 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. Through specialized treatment, this product is devoid of DNA residues and is appropriate for the development of biopharmaceutical - related detection reagents.

Size

200 U/μL

Components

1.200 U/μL Neoscript RTase HS Ⅱ (NA) (DG)

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 Concentration
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 Concentration
5×First-Strand Buffer	4 μL
200 U/μL Neoscript RTase HS Ⅱ (NA) (DG)	1 μL
40 U/μL RNase Inhibitor (DG)	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 HS II (NA) (DG) 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) (DG)	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.