NGScript III All-in-one RT Mix with（gDNA）

Code No.： NG049M 100 T

Introduction：

This product is a one-tube premix for genomic DNA removal and reverse transcription, enabling simultaneous completion of genomic DNA removal and cDNA synthesis in just 5 minutes with a single step, effectively avoiding the risk of sample contamination and RNA degradation caused by complex pipetting. It contains a highly efficient third-generation reverse transcriptase, which, when used with the optimized 5×NGScript III All-in-one RT Buffer, ensures consistent reverse transcription efficiency across different RNA template concentrations, making it particularly suitable for the synthesis of short cDNA fragments. The buffer contains an optimized ratio of Random Primer and Oligo18(dT), eliminating the need for separate primer addition and simplifying the workflow. The reverse transcription products are compatible with both dye-based and probe-based qPCR for subsequent gene expression analysis.

Product Specifications：

1. Minimal operation: Reverse transcription and genomic removal are completed in one step, avoiding RNA sample degradation while reducing the risk of contamination;
2. Ultra-high efficiency: Highly efficient reverse transcriptase and optimized reagent ratios ensure consistent reverse transcription efficiency across different RNA template concentrations;
3. Wide applicability: The reaction system can effectively expand the complex template structure and high GC content template.

Content

content	100 T
NGScript III All-in-one RT Mix	100 μl
5×NGScript III All-in-one RT Buffer	400 μl
No RT Control Mix	10 μl
Water nuclease-free	1.5 ml

No RT Control Mix does not contain the RT enzyme, and is used to check for residual genomic DNA in the RNA.

Storage： Store at -20 ℃ for up to two years and avoid repeated freezing and thawing.

Precautions:

1. Please avoid RNase contamination during the experiment.
2. This product uses a heat-sensitive DNase; please keep the NGScript III An-one RT Mix, 5×NGScript III All-in-one RT Buffer, and No RT Control Mix on ice.
3. Before use, briefly centrifuge the components to collect the bottom of the tube. Gently pipette up and down to mix thoroughly before accurately aspirating to prevent uneven concentration affecting the experimental results.
4. The integrity of the RNA plays a decisive role in cDNA synthesis efficiency; therefore, please select a reliable RNA extraction/purification method.
5. For a 20 μl reverse transcription reaction, it is recommended to add no more than 1 μg of total RNA.
6. The undiluted cDNA product from reverse transcription can be used directly as a template for qPCR. It is recommended that the volume of the cDNA product does not exceed 1/10 of the qPCR reaction volume.
7. RNA can be stored loge term at -70 ℃ or below, and cDNA synthesis products can be stored at -20 ℃.

Operation Procedure:

1. Preparation of reaction system

content	20 μl
RNA	≤1 μg total RNA or≤0.1 μg poly(A) mRNA
NGScript III All-in-one RT Mix	1 μl
5×NGScript III All-in-one RT Buffer	4 μl
Water nuclease-free	Up to 20 μl

After gently pipetting up and down to mix thoroughly, perform a brief centrifugation.

2. No RT Control raction（Optional）

This reaction is a negative control without reverse transcriptase, used to check for residual genomic DNA in the RNA template.

content	20 μl
RNA	≤1 μg total RNA or≤0.1 μg poly(A) mRNA
No RT Control Mix	1 μl
5×NGScript III All-in-one RT Buffer	4 μl
Water nuclease-free	Up to 20 μl

After gently pipetting up and down to mix thoroughly, perform a brief centrifugation.

3. reaction conditions

Temperature	Time
37 ℃	2 min
50 ℃	5-15 min
85 ℃	2 min

For complex templates, a temperature of 55-60 ℃ can be used to improve reverse transcription efficiency. The reverse transcription time can be adjusted appropriately.

4. Please place the resulting cDNA on ice for subsequent experiments or store it frozen.