HiScript® III Reverse Transcriptase

Cat# NB-54-0181 size: 10000U

Product Description

HiScript III Reverse Transcriptase is an upgraded version of Hiscript II Reverse Transcriptase that designed for efficient reverse transcription reactions at 37℃. HiScript III Reverse Transcriptase still retains the thermostability of Hiscript II Reverse Transcriptase. For RNA with complex secondary structure, the reverse transcription reaction temperature can be raised to 50 ~ 55℃, avoiding the inhibitory effect of complex secondary structure on cDNA synthesis. In addition, this product still has superior continuous synthesis ability and superior impurity tolerance.

Components

5 × HiScript III Buffer	500 µl
HiScript III Reverse Transcriptase (200 U/µl)	50 µl

Storage

Store at -30 ~ -15°C, transport at ≤0°C

Applications

It is applicable for reverse transcription of animal, plant and microbial RNA.

Source

It is cloned from M-MLV with improved reverse transcriptase gene and purified from E.coli.

Unit Definition

One unit (U) is defined as the amount of enzyme that incorporates 1 nmol of dTTP into acid-insoluble material in 10 min at 37℃ with Poly (rA)·Oligo (dT) as the template/primer.

Notes

Prevent RNase contamination

Keep the experiment area clean. Wear disposable gloves and masks, and use RNase-free tubes and tips.

Primer selection

1. If cDNA prodcucts will be used for PCR

- For eukaryotic RNA tempaltes, use Oligo dT primer to obtain the highest yield of full-length cDNA.
- Use gene-specific primer (GSP) can obtain the highest specificity. However, switch to Oligo dT or random hexamers if GSP fails in 1st strand cDNA synthesis.
- Random hexamers have the lowest specificity and it can be used for RNA templates, including mRNA, rRNA, and tRNA. Use random hexamers when Oligo dT or GSP fails in cDNA synthesis due to complex secondary structure, high GC content, or prokaryotic RNA templates.

2. If cDNA prodcucts will be used for qPCR

- Use the mixture of Oligo dT and random hexamers. In this way, the cDNA synthesis efficiency of each region of the mRNA can be the same, which helps to improve the authenticity and repeatability of the quantitative results.

Experiment Process

PCR

1. RNA Denaturation*

Mix the following components in a RNase-free PCR tube:

RNase free ddH_2O	to 10 µl
Total RNA	10 pg - 5 µg
or Poly A^+ RNA	10 pg - 500 ng

Incubate at 65℃ for 5 min and then chill on ice immediately for 2 min.

* RNA denaturation benifits the cDNA yield. However, for cDNA >3 kb, please do not skip the denaturation step.

2. Preparation of 1st strand cDNA synthesis reaction mixture

Mixture of Step 1	10 µl
5 × HiScript III Buffer	4 µl
dNTP Mix (10 mM each)	1 µl
HiScript III Reverse Transcriptase (200 U/µl)	1 µl
RNase inhibitor (40 U/µl)	1 µl
Oligo $(dT)_{20}VN$ (50 µM)	
or Random Hexamers (100 µM)	1 µl
or Gene Specific Primers (2 µM)	
RNase-free ddH_2O	2 µl

Mix gently with a pipette.

3. Run the following program for 1st strand cDNA synthesis

25°C[a]	5 min
37°C[b]	45 min
85°C	5 sec

a. This step is required only when using the Random hexamers. Please omit this step when using Oligo (dT)20VN or Gene Specific Primer.
b. For templates with complex secondary structure or high GC content, the temperature can be increased to 55°C, which will benefit the yield.

The products can be used for PCR immediately or be stored at -20°C for 6 months. However, it is recommended to store at -70°C after aliquoting for long term storage. Avoid repeated freezing and thawing.

qPCR

1. Prepare the 1st strand cDNA reaction mix

Mix the following components in a RNase-free PCR tube:

RNase free ddH_2O	to 20 µl
5 × HiScript III Buffer	4 µl
dNTP Mix (10 mM each)	1 µl
HiScript III Reverse Transcriptase (200 U/µl)	1 µl
RNase inhibitor (40 U/µl)	1 µl
Oligo $(dT)_{20}VN$ (50 µM)	1 µl
Random hexamers (100 µM)	1 µl
Total RNA	10 pg - 1 µg
or Poly A^+ RNA	10 pg - 100 ng

Mix gently with a pipette.

2. Run the following program for 1st strand cDNA synthesis

37°C*	15 min
85°C	5 sec

* For templates with complex secondary structure or high GC content, the temperature can be increased to 55°C, which will benefit the yield.

The products can be used for qPCR immediately or be stored at -20°C for 6 months. However, it is recommended to store at -70°C after aliquoting for long term storage. Avoid repeated freezing and thawing.