HiScript® II Reverse Transcriptase

R201

Version 21.1

Product Description

HiScript II Reverse Transcriptase is a new generation reverse transcriptase optimized from the M-MLV (RNase H-) Reverse Transcriptase. Compared with the last generation reverse transcriptase, the thermo-stability of this product is significantly improved. The half-life of HiScript II Reverse Transcriptase at 50℃ is >240 min. At 55℃, it can also be stable for a long time, which significantly benifits the transcription of RNA templates with complex secondary structure. In addition, it has a improved template affinity and cDNA synthesis efficiency. It has a good resistance to most RT-PCR inhibitors and is suitable for long-fragment cDNA amplification (as long as 20 kb).

Components

Components	R201-01 2,000 U	R201-02 10,000 U
5 × HiScript II Buffer	500 µl	500 µl
HiScript II Reverse Transcriptase (200 U/µl)	10 µl	50 µl

Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

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 13 µl
Oligo $(dT)_{23}VN$ (50 µM)	
or Random hexamers (50 ng/µl)	1 µl
or Gene Specific Primers (2 µM)	
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	13 µl
5 × HiScript II Buffer	4 µl
dNTP Mix (10 mM each)	1 µl
HiScript II Reverse Transcriptase (200 U/µl)	1 µl
RNase inhibitor (40 U/µl)	1 µl

Mix gently with a pipette.

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

25℃[a]	5 min
50℃[b]	45 min
85℃	2 min

a. It is necessary when using random hexamers. Please skip this step when using Oligo $(dT)_{23}VN$ or GSP.

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

The products can be used for PCR immediately or be stored at -20℃ for 6 months. However, it is recommended to store at -70℃ 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 II Buffer	4 µl
dNTP Mix (10 mM each)	1 µl
HiScript II Reverse Transcriptase (200 U/µl)	1 µl
RNase inhibitor (40 U/µl)	1 µl
Oligo $(dT)_{23}VN$ (50 µM)	1 µl
Random hexamers (50 ng/µl)	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

25℃	5 min
50℃*	15 min
85℃	2 min

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

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