HiScript II Reverse Transcriptase (Glycerol-free)

RL201

Version 23.1

Product Description

HiScript II Reverse Transcriptase (Glycerol-free) is a lyophilizable version of HiScript II Reverse Transcriptase (Vazyme #R201). It can be applied to downstream lyophilization technology while maintaining the great reverse transcription performance and stability of Vazyme #R201. This product does not contain excipients, please add your own as needed.

Components

	Components	RL201-01 (10,000 U)	RL201-02 (40,000 U)
■	5 × HiScript II Buffer	200 µl	800 µl
■	HiScript II Reverse Transcriptase(Glycerol-free) (200 U/µl)	50 µl	200 µl

Storage

Store at -30 ~ -15°C and transport at ≤0°C.

Applications

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

Source

A recombinant *E. coli* strain carrying modified M-MLV (H-) reverse transcriptase gene.

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°C, with Poly(rA)·Oligo (dT) as the template/primer.

Notes

For research use only. Not for use in diagnostic procedures.

Prevent RNase contamination

Please keep the experiment area clean; Wear disposable gloves and masks; Use RNase-free consumables such as centrifuge tubes and pipette tips.

Primer selection

1. For PCR

- For eukaryotic RNA templates, use Oligo dT primer to obtain the highest yield of full-length cDNA by pairing with 3' Poly A of eukaryotic mRNA.
- Gene Specific Primers (GSP) has the highest specificity. If GSP fails in the 1st strand cDNA synthesis, Oligo $(dT)_{20}VN$ or Random hexamers can be used for reverse transcription.
- Random hexamers have the lowest specificity. All RNA, including mRNA, rRNA and tRNA can be used as the template of Random hexamers. Random hexamers can be used as primers, when Oligo $(dT)_{20}VN$ or GSP can not effectively guide cDNA synthesis for the target region has complex secondary structure and high GC content, or the template is prokaryotic origin.

2. 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

◇ **For PCR**

1. RNA Denaturation*

Components	Volume
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°C for 5 min and then chill on ice immediately for 2 min.

* The denaturation step helps to open the secondary structures to improve the first strand cDNA yield. For cDNA fragment longer than 3 kb, please do not ignore the denaturation step.

2. Preparation of reaction solution for 1st strand cDNA synthesis

Components	Volume	
Mixture of Step 1	13 μl	
5 × HiScript II Buffer	4 μl	■
dNTP Mix (10 mM each)	1 μl	
HiScript II Reverse Transcriptase (Glycerol-free) (200 U/μl)	1 μl	■
RNase inhibitor (40 U/μl)	1 μl	

Gently pipette up and down several times to mix thoroughly.

3. Reaction Program

Temperature	Time
25°C[a]	5 min
50°C[b]	45 min
85°C	2 min

a. This step is required only when using the Random hexamers. Please omit this step when using Oligo $(dT)_{20}$VN or GSP.

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 product can be used for PCR immediately or be stored at -20°C for 6 months. It is recommended to store in aliquots at -70°C for long term storage. cDNA should avoid repeated freezing and thawing.

◇ **For qPCR**

1. Preparation of reaction solution for 1st strand cDNA synthesis

Mix the following components in an RNase-free centrifuge tube:

Components	Volume	
RNase-free ddH_2O	to 20 μl	
5 × HiScript II Buffer	4 μl	■
dNTP Mix (10 mM each)	1 μl	
HiScript II Reverse Transcriptase (Glycerol-free) (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	

Gently pipette up and down several times to mix thoroughly.

2. Reaction Program

Temperature	Time
25°C	5 min
50°C*	15 min
85°C	2 min

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

The product can be used for qPCR immediately or be stored at -20°C for 6 months. It is recommended to store in aliquots at -70°C for long term storage. cDNA should avoid repeated freezing and thawing.