HiScript II 1st Strand cDNA Synthesis Kit (+gDNA Wiper)

Catalog # R212

Version 7.0

Vazyme biotech co., ltd.

Introduction

The Vazyme HiScript II Reverse Transcriptase (+gDNA Wiper) is designed for the 1st strand cDNA synthesis with genomic DNA removal treatments. The HiScript II Reverse Transcriptase is a new generation reverse transcriptase optimized from the M-MLV (RNase H-) Reverse Transcriptase. The half-life of HiScript II at 50℃ is > 240 min. Even at 55℃, the HiScript II can stay stable for a long time, which significantly benifits the transcription of RNA templates with complex secondary structures. In addition, the HiScript II 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).

The residual genomic DNA in RNA template can be removed rapidly and completely after a treatment (42℃ for 2 min) with the 4× gDNA Wiper. The 10× RT Mix contains an optimized buffer and dNTPs. The HiScript II Enzyme Mix contains the HiScript II Reverse Transcriptase and the RNase inhibitor. The Oligo-$(dT)_{23}$VN has a better affinity to Ploy A^+ RNA than Oligo-$(dT)_{18}$. In addition, random hexamers and gene-specific primers (GSP) are also optional.

Contents of Kits

Components	R212-01 50 rxn (20 μl/rxn)	R212-02 100 rxn (20 μl/rxn)
RNase free ddH_2O	1 ml	1 ml
4× gDNA Wiper Mix	200 μl	400 μl
10× RT Mix [a]	100 μl	200 μl
HiScript II Enzyme Mix [b]	100 μl	200 μl
Oligo-$(dT)_{23}$VN (50 μM)	50 μl	100 μl
Random Hexamers (50 ng/μl)	50 μl	100 μl

a. contains dNTPs.

b. contains RNase inhibitor.

Storage

All the components should be stored at -20℃.

Protocol

Note: 1. Use high quality total RNA with high intergrity for reverse transcription.

2. To avoid RNase contamination, please keep the experiment area clean, wear clean gloves and masks, and use RNase-free tubes and tips.

3. **Primer selection** (Oligo-$(dT)_{23}$VN, Random hexamers, or GSP)

If the cDNA prodcuct will be used for PCR

- For eukaryotic RNA tempaltes, generally, use Oligo-$(dT)_{23}$VN to obtain the highest yield of full-length cDNA.
- Use gene-specific primer (GSP) to obtain the highest specificity. However, switch to Oligo-$(dT)_{23}$VN or random hexamers if GSP fails in the 1st-strand cDNA synthesis.
- Random hexamers with the lowerst specificity can be used for RNA templates, including mRNA, rRNA, and tRNA. Use random hexamers when Oligo-$(dT)_{23}$VN or GSP fails in cDNA synthesis due to complex secondary structure, high GC content, or prokaryotic RNA template.

If the cDNA prodcuct will be used for qPCR

- Use the mixture of Oligo-$(dT)_{23}$VN or random hexamers.

1. If the cDNA prodcuct will be used for PCR

1.1. RNA Denaturation*

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

RNase free ddH_2O	to 12 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.

Note: * RNA denaturation benifits the cDNA yield. However, for cDNA < 3 kb, please skip the denaturation step.

1.2. Removal of Genomic DNA

Add 4 μl of 4× gDNA Wipter to the mixture of **Step 1.1** (12 μl), mix thoroghtly, and incubate at 42℃ for 2 min.

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

Mixture of **Step 1.2.**	16 µl
10× RT Mix	2 µl
HiScript II Enzyme Mix	2 µl

1.4. Start the 1st-strand cDNA synthesis.

25℃*	5 min
50℃**	45 min
85℃	2 min

Note: * Only necessary when using random hexamers. Please skip this step when using Oligo-$(dT)_{23}$VN or Gene Specific Primers (GSP).
** 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 stored at -80℃ and make aliquots to avoid repeated freezing and thawing.

2. If the cDNA prodcuct will be used for qPCR

2.1. Removal of Genomic DNA

Mix the following components in a RNase-free microtube by pipetting, and incubate at 42℃ for 2 min.

RNase free ddH_2O	to 16 µl
4× gDNA Wiper Mix	4 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

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

Mixture of **Step 1.2.**	16 µl
10× RT Mix	2 µl
HiScript II Enzyme Mix	2 µl

2.3. Start the 1st-strand cDNA synthesis.

25℃	5 min
50℃*	15 min
85℃	2 min

Note: * 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 stored at -80℃ and make aliquots to avoid repeated freezing and thawing.