HiScript II 1st Strand cDNA Synthesis Kit

Catalog # R211

Version 7.0

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Introduction

The Vazyme 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 Vazyme HiScript II 1st Strand cDNA Synthesis Kit contains all the components necessary for the 1st strand cDNA synthesis. The products are suitable for PCR and qPCR. The 2× 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	R211-01 50 rxn (20 μl/rxn)	R211-02 100 rxn (20 μl/rxn)
RNase free ddH_2O	1 ml	1 ml
2× RT Mix [a]	500 μl	1 ml
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 8 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. Mix the following components in a RNase-free PCR tube by gently pipetting:

Mixture of **Step 1.1.**	8 μl
2× RT Mix	10 μl
HiScript II Enzyme Mix	2 μl

1.3. Start the 1st-strand cDNA synthesis.

25℃*	5 min
50℃**	45 min
85℃	5 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. Mix the following components in a RNase-free PCR tube by pipetting:

RNase free ddH_2O	to 20 μl
2× RT Mix	10 μl
HiScript II Enzyme Mix	2 μl
Oligo-$(dT)_{23}$ VN (50 μM)	1 μl
Random hexamers (50 ng/μl)	1 μl
Total RNA	1 pg-1 μg
or Poly A^+ RNA	10 pg-100 ng

2.2. 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.