HiScript III 1st Strand cDNA Synthesis Kit (+gDNA wiper)

R312

Version 23.1

Product Description

HiScript III 1st Strand cDNA Synthesis Kit (+gDNA wiper) is an upgraded version of HiScript II 1st Strand cDNA Synthesis Kit (+gDNA wiper), including a new generation of reverse transcriptase HiScript III Reverse Transcriptase and Buffer optimized for reverse transcription, further improving the efficiency of 1st strand synthesis. The 5 × gDNA wiper Mix in the kit can quickly remove genomic DNA contamination within 2 min, which makes the results more reliable and simplifies the process of qPCR primer design without the need of spanning an exon-exon junction. The kit contains single-component reverse transcription primers Oligo $(dT)_{20}VN$ and Random hexamers, which allows users to choose primers flexibly for subsequent experiments as required. The kit can be used to synthesize full-length cDNA (up to 20 kb) for cloning and other downstream experiments, as well as highly uniform cDNA for qPCR.

Components

	Components	R312-01 50 rxns (20 μl/rxn)	R312-02 100 rxns (20 μl/rxn)
☐	RNase-free ddH_2O	1 ml	1 ml
	5 × gDNA wiper Mix	100 μl	200 μl
	10 × RT Mix[a]	100 μl	200 μl
	HiScript III Enzyme Mix[b]	100 μl	200 μl
	Oligo $(dT)_{20}VN$	50 μl	100 μl
	Random hexamers	50 μl	100 μl

a. It contains dNTPs.

b. It contains RNase inhibitor.

Storage

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

10 × RT Mix contains high concentration of DTT, which may precipitate at low temperature. Please restore to room temperature, shake gently and mix thoroughly, wait to redissolve the precipitation before use.

Applications

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

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.

Primers selection

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.

For qPCR

- Mixing Oligo $(dT)_{20}VN$ with Random hexamers in a recommended ratio enables the same efficiency of cDNA synthesis in each region of the mRNA, which helps to improve the authenticity and reproducibility of quantitative results.
- Reverse transcription can be performed directly without the genome removal step, and the spare volume can be filled with RNase-free ddH_2O.

Experiment Process

◇ **For PCR**

1. RNA Denaturation*

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

RNase-free ddH_2O	to 8 μl	☐
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. Removal of genomic DNA

Mixture of Step 1	8 μl	
5 × gDNA wiper Mix	2 μl	■

Gently pipette up and down several times to mix thoroughly. Incubate at 42°C for 2 min.

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

Mixture of Step 2	10 μl	
10 × RT Mix	2 μl	■
HiScript III Enzyme Mix	2 μl	■
Oligo $(dT)_{20}VN$	1 μl	■
or Random hexamers		■
RNase-free ddH_2O	5 μl	□

Gently pipette up and down several times to mix thoroughly.

▲This product is also suitable for reverse transcription using GSP. To avoid the potential effect of gDNA wiper on GSP, please add GSP (2 pmol) to the mixure.

4. Reaction Program

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

a. Only necessary when using Random hexamers. Please skip this step when using Oligo $(dT)_{20}VN$ or GSP.

b. For template with complicated secondary structures or high GC content, the temperature can be increased to 50°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. Removal of genomic DNA

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

RNase-free ddH_2O	to 10 μl	□
5 × gDNA wiper Mix	2 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. Incubate at 42°C for 2 min.

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

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

Mixture of Step 1	10 μl	
10 × RT Mix	2 μl	■
HiScript III Enzyme Mix	2 μl	■
Oligo $(dT)_{20}VN$	1 μl	■
Random hexamers	1 μl	■
RNase-free ddH_2O	4 μl	□

Gently pipette up and down several times to mix thoroughly.

▲This product is also suitable for reverse transcription using GSP. To avoid the potential effect of gDNA wiper on GSP, please add GSP (2 pmol) to the mixture.

3. Reaction Program

37°C*	15 min
85°C	5 sec

*For template with complicated secondary structures or high GC content, the temperature can be increased to 50°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.