GB-Script™ III 1st Strand cDNA Synthesis Kit (+ gDNA wiper)

R312 Version 21.1

Product Description

GB-Script™ III (also referred to as 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 at 42°C for 2 min, which makes the results more reliable and simplifies qPCR primer design process without the need to design primers across introns. 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 temperatures. Please restore to room temperature, shake gently and mix thoroughly, wait to dissolve the precipitation again before use.

Applications

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

Notes

Prevent RNase contamination

Please keep the experiment area clean; Wear clean gloves and masks, and use new centrifuge tubes, tips and other supplies to ensure experiment is RNase-free.

Primers selection

For PCR

- For eukaryotic RNA templates, generally, use Oligo $(dT)_{20}VN$ to obtain the highest yield of full-length cDNA.
- Use gene specific primer (GSP) to obtain the highest specificity. However, switch to Oligo $(dT)_{20}VN$ or Random hexamers if GSP fails in the 1st strand cDNA synthesis.
- Random hexamers have the lowest specificity. All RNA, including mRNA, rRNA and tRNA can be templates of Random hexamers. Random hexamers may be used when the target region has complex secondary structures or high GC content, or when the template is derived from prokaryote and Oligo $(dT)_{20}VN$ or Gene Specific Primes (GSP) can not effectively guide cDNA synthesis.

For qPCR

- Mixing Oligo $(dT)_{20}VN$ with Random hexamers according to the guide ratio can make the synthesis efficiency of cDNA in each region of mRNA equal, which helps to improve the authenticity and reproducibility of quantitative results.

Experiment Process

◇ If the product will be used for PCR

1. RNA Denaturation*

Mix the following components in a 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, place on ice rapidly and let rest 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, do not ignore the denaturation step.

2. Removal of genomic DNA

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

Mixture of previous step	8 μl
5 × gDNA wiper Mix	2 μl

Mix gently with a pipette. Incubate at 42°C for 2 min.

3. Preparation of 1st strand cDNA synthesis reaction mixture

Mixture of previous step	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

Mix gently with a pipette.

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

4. Perform the 1st strand cDNA synthesis reaction under the following conditions

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 Gene Specific Primer.

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 directly be used for PCR reactions, or store at -20°C and used within six months. It is recommended to store in aliquots at -70°C for a long-term storage, and cDNA should be avoided repeated freezing and thawing.

◇ If the product will be used for qPCR

1. Removal of genomic DNA

Mix the following components in a 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

Mix gently with a pipette. Incubate at 42°C for 2 min.

2. Preparation of 1st strand cDNA synthesis reaction mixture

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

Mixture of previous step	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

Mix gently with a pipette.

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

3. Perform the 1st strand cDNA synthesis reaction under the following conditions

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

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