

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

R312

Version 22.2



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)₂₀VN 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)
<input type="checkbox"/> RNase-free ddH ₂ O	1 ml	1 ml
<input checked="" type="checkbox"/> 5 × gDNA wiper Mix	100 µl	200 µl
<input checked="" type="checkbox"/> 10 × RT Mix ^a	100 µl	200 µl
<input checked="" type="checkbox"/> HiScript III Enzyme Mix ^b	100 µl	200 µl
<input checked="" type="checkbox"/> Oligo (dT) ₂₀ VN	50 µl	100 µl
<input checked="" type="checkbox"/> 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

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)₂₀VN 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)₂₀VN 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)₂₀VN 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 ddH₂O.

Experiment Process

◇ For PCR

1. RNA Denaturation*

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

RNase-free ddH ₂ O	to 8 µl	<input type="checkbox"/>
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 ₂ O	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 mixture.

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)₂₀VN 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 ₂ O	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 ₂ O	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.

