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

#Cat: NB-54-0366-01 **Size: 50rxns**
#Cat: NB-54-0366-02 **Size: 100rxns**

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

Product Description

HiScript IV 1st Strand cDNA Synthesis Kit (+gDNA wiper) is the fourth generation of the HiScript series for synthesizing first strand cDNA using Total RNA or Poly A^+ RNA as templates. Compared to previous versions, the HiScript IV 1st Strand cDNA Synthesis Kit (+gDNA wiper) further enhances reverse transcription efficiency, including stronger extension ability, faster reaction speed and higher tolerance to inhibitors, especially suitable for downstream qPCR quantification and long fragment cDNA amplification. This product premixes HiScript IV RTase, RNase inhibitor, dNTP Mix, etc., and only appropriate reverse transcription primers need to be added according to downstream experiments. The kit provides a genomic DNA elimination module, where the 5 × gDNA wiper Mix in the kit can quickly remove genomic DNA contamination under conditions of 42°C for 2 min, ensuring reliable and authentic experimental data.

Components

Components	*NB-54-0366* 50 rxns (20 µl/rxn)	*NB-54-0366* 100 rxns (20 µl/rxn)
RNase-free ddH2O	1 ml	1 ml
5 × gDNA wiper Mix	100 µl	200 µl
4 × HiScript IV RT SuperMix*	250 µl	500 µl
Oligo (dT)20VN	50 µl	100 µl
Random Primers	100 µl	200 µl
4 × No RT Control Mix	25 µl	

* It contains dNTP Mix and RNase inhibitor.

Storage

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

Applications

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

Notes

For research use only. Not for use in diagnostic procedures.

Neo Biotech
74 rue des Suisses – 92000 Nanterre

◇ **Prevent RNase contamination**

Please keep the experiment area clean; Wear disposable gloves and masks; Use of RNase-free consumables, such as centrifuge tubes and pipette tips.

◇ **Primer selection**

For PCR

● For eukaryotic RNA templates, use Oligo (dT)20VN primer to obtain the highest yield of full-length cDNA by pairing with 3' end poly(A) tail 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 Primers at the indicated ratios results in the same efficiency of cDNA synthesis in all regions of the mRNA, which contributes to the authenticity and reproducibility of the quantitative results.

● The Oligo (dT)20VN and Random Primers provided in this kit have been specifically optimized. Use of reverse transcription primers from other sources may result in decreased reverse transcription efficiency.

● Reverse transcription can be performed directly without a genome elimination step, and the empty volume can be made up with RNase-free ddH2O.

Experiment Process

◇ **Subsequent experiment is PCR**

◇ **1. RNA Denaturation**

RNase-free ddH2O	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.

▲ Intact RNA is a prerequisite for the synthesis of long fragment cDNAs. If RNA integrity cannot be guaranteed, it is recommended to increase the amount of RNA input to increase the number of intact RNA copies. This can be increased to a maximum of 5 µg.

2. gDNA Elimination

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

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

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

Mixture of Step 2	10 µl
4 × HiScript IV RT SuperMix	5µl
Total RNA	1µl
or Poly A + RNA	4µl

Gently pipette up and down several times to mix thoroughly.
* Alternatively, GSP (2 pmol) or Random Primers can be used for reverse transcription.

4. Reaction Program

50°C	5 min*
85°C	5 sec

* This reagent can synthesize 15 kb cDNA within 5 min. If more cDNA products are required, the reaction time can be extended to 30 min.

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.

◇ **Subsequent experiment is qPCR**

1. gDNA Elimination

RNase-free ddH2O	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. 42°C 2 min。

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

Mixture of Step	10 µl
4 × HiScript IV RT SuperMix	5 µl
Oligo (dT)20VN	1 µl
Random Primers	2 µl
RNase-free ddH2O	2 µl

Gently pipette up and down several times to mix thoroughly.
* Alternatively, GSP (2 pmol) can be used for reverse transcription.

3. Reaction Program

37°C	15 min
85°C	5 sec

* For templates with complex secondary structure 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.

◇ **No RT Control (optional)**

No RT Control refers to the reverse transcription negative control reaction without the addition of reverse transcriptase, used to test for the presence of genomic DNA residues and genome elimination in the RNA template

Mixture without gDNA*	10µl
4× No RT Control Mix	5µl
RNase-free ddH2O	5µl

Gently pipette up and down several times to mix thoroughly. 10 µl 5 µl 5 µl

*If the subsequent experiment is PCR, this refers specifically to the mixture at the end of step 2 in the PCR experimental process; if the subsequent experiment is qPCR, this refers specifically to the mixture at the end of step 1 in the qPCR experimental process.

Reaction Program

37°C/50°C*	15 min
85°C	5 sec

* Select the appropriate reaction temperature according to the application scenario: if the subsequent experiment is PCR, 50°C is recommended; if the subsequent experiment is qPCR, 37°C is recommended.

The product can be used for PCR/qPCR immediately or be stored at -20°C