HiScript II Reverse Transcriptase

R201

Version 22.1

Product Description

HiScript II Reverse Transcriptase is a new reverse transcriptase obtained by in vitro molecular evolution technology on the basis of M-MLV(RNase H-)Reverse Transcriptase. Compared with the previous generation of HiScript Reverse Transcriptase, HiScript II Transcriptase has greatly improved thermal stability, with a half-life of more than 4 h at 50℃, and can remain stable for a long time at 55℃. It is suitable for reverse transcription of RNA templates with complex secondary structures for complex secondary structures.In addition, HiScript II Transcriptase adds multiple point mutations, which further enhances the template affinity and progression, which greatly improves the synthesis ability of full-length cDNA, and can obtain cDNA up to 20 kb. It has higher tolerance to common reverse transcription inhibitors and is very suitable for reverse transcription reaction of plant tissue RNA rich in polysaccharides and polyphenols.

Components

Components	R201-01 2,000 U	R201-02 10,000 U
5 × HiScript II Buffer	500 μl	500 μl
HiScript II Reverse Transcriptase (200 U/μl)	10 μl	50 μl

Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

Applications

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

Source

A recombinant *E. coli* strain carrying an improved reverse transcriptase gene cloned from M-MLV.

Unit definition

One unit (U) is defined as the amount of enzyme that incorporates 1 nmol of dTTP into acid-insoluble material in 10 min at 37℃ with Poly(rA)-Oligo (dT) as the template/primer.

Notes

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

Prevent RNase contamination

Please keep the experimental area clean; wear clean gloves and masks during operation; consumables such as centrifuge tubes and pipette tips used in the experiment must be RNase-free.

Choose the primer

1. Follow-up experiment is PCR

- If the template is of eukaryotic origin, Oligo dT is generally preferred, which can be paired with the 3' Poly A tail of eukaryotic mRNA to obtain the highest yield of full-length cDNA.
- Use gene-specific primer (GSP) can obtain the highest specificity. However, switch to Oligo dT or random hexamers if GSP fails in 1st strand cDNA synthesis.
- Random hexamers have the lowest specificity, and all RNAs, including mRNA, rRNA, and tRNA, can be used as templates for random hexamers. When the target region has complex secondary structure or high GC content, or the template is of prokaryotic origin, and Oligo dT or gene-specific primers (GSP) cannot effectively guide cDNA synthesis, Random hexamers can be used as primers.

2. Follow-up experiment is qPCR

- Mixing Oligo dT with Random hexamers can make the cDNA synthesis efficiency of each region of mRNA the same, which helps to improve the authenticity and reproducibility of quantitative results.

Experiment Process

◇Follow-up experiment is PCR

1. RNA Template denaturation*

Prepare the following mixtures in RNase-free centrifuge tubes:

Component	Volume
RNase-free ddH₂O	to 13 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.

* RNA template denaturation helps to open secondary structure, which can greatly improve the yield of first-strand cDNA. Do not omit the denaturation step for cDNA fragments longer than 3 kb.

2. Prepare the first-strand cDNA synthesis reaction solution

Component	Volume	
Mixture from the previous step	13 μl	
5 × HiScript II Buffer	4 μl	■ (red)
dNTP Mix (10 mM each)	1 μl	
HiScript II Reverse Transcriptase (200 U/μl)	1 μl	■ (blue)
RNase inhibitor (40 U/μl)	1 μl	

Gently pipette up and down several times to mix thoroughly.

3. Carry out the first-strand cDNA synthesis reaction according to the following conditions

Temperature	Time
25℃[a]	5 min
50℃[b]	45 min
85℃	2 min

a. This step is only required when using Random hexamers; omit this step when using Oligo (dT)$_{23}$VN or Gene Specific Primer.
b. If the template has complex secondary structure or high GC region, the reaction temperature can be increased to 55℃ to help increase the yield.

The product can be used for PCR reaction immediately, or stored at -20℃ and used within half a year; for long-term storage, it is recommended to store at -70℃ after aliquoting. Avoid repeated freeze-thaw of cDNA.

◇ Follow-up experiment is qPCR

1. Prepare the first-strand cDNA synthesis reaction solution

Mix the following components in RNase-free centrifuge tubes:

Component	Volume	
RNase-free ddH₂O	to 20 μl	
5 × HiScript II Buffer	4 μl	■ (red)
dNTP Mix (10 mM each)	1 μl	
HiScript II Reverse Transcriptase (200 U/μl)	1 μl	■ (blue)
RNase inhibitor (40 U/μl)	1 μl	
Oligo (dT)$_{23}$VN (50 μM)	1 μl	
Random hexamers (50 ng/μl)	1 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.

2. Run the following program for 1st strand cDNA synthesis

Temperature	Time
25℃	5 min
50℃*	15 min
85℃	2 min

* If the template has complex secondary structure or high GC region, the reaction temperature can be increased to 55℃ to help increase the yield.

The product can be used for qPCR reaction immediately, or stored at -20℃ and used within half a year; it is recommended to store at -70℃ after aliquoting for long-term storage. Avoid repeated freeze-thaw of cDNA.