HiScript® III Reverse Transcriptase

R302-01

Version 8.1

Vazyme biotech co., ltd.

Introduction

Hiscript® III Reverse Transcriptase is an upgraded version of Hiscript® II Reverse Transcriptase that designed for efficient reverse transcription reactions at 37˚C. Hiscript® III Reverse Transcriptase still retains the thermostability of the second-generation Hiscript II Reverse Transcriptase. The enzyme is active up to 50˚C, providing higher specificity, higher yield of cDNA and more full-length cDNA product, which is very helpful when dealing with RNA that contains high amounts of secondary structure. In addition, this product still has superior continuous synthesis ability and superior impurity tolerance.

Package Information

Components	R302-01 10,000 U
5x HiScript III Buffer	500 µl
HiScript III Reverse Transcriptase (200 U/ µl)	50 µl

Storage

Store at -30˚C to -15˚C. Transportation condition is -20˚C to 0˚C.

Unit Definition

One activity unit (U) is defined as the amount of enzyme that will incorporate 1 nmol of dttp into acid-insoluble material in 10 minutes at 37˚C using Poly (rA)•Oligo (dT) as template-primer.

Unit Definition

Exonuclease Activity: Incubation of 200 U of this enzyme with 0.6 µg λ-Hind III at 37˚C for 1 hour results in no change on electrophoretic bands.

Endonuclease Activity: Incubation of 200 U of this enzyme with 0.3 µg pBR 322 DNA for 4 hour at 37˚C results in no change on electrophoretic bands.

Contamination of Rnase: Incubation of 200 U of this enzyme with 1 µg of 293 cell RNA for 30 min at 30˚C results in no change on electrophoretic bands.

Functional Test:

1. A single 4.6 kb VIN gene was identified after agarose gel electrophoresis and EB staining from 1µg HeLa cell total RNA as template and Oligo $(dT)_{20}$ as primer incubated for 45 minutes at 37˚C using 200 U of HiScript® III Reverse Transcriptase.
2. A single 550 bp GAPDH gene was identified after agarose gel electrophoresis and EB staining from 1 pg Hela cell total RNA as template and Oligo $(dT)_{20}$ as primer incubated for 30 minutes at 37˚C using 200 U of HiScript® III Reverse Transcriptase.
3. A single 7.1 kb (GC-rich) POLE gene was identified after agarose gel electrophoresis and EB staining from 500 ng Hela cell total RNA as template and Oligo $(dT)_{20}$ as primer incubated for 45 minutes at 37˚C using 200 U of HiScript® III Reverse Transcriptase.

Notes

Prevent RNase contamination

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.

Choosing Primers for PCR

- Oligo dT VN hybridizes at high efficiency to the 3´ poly(A) region found in most mature eukaryotic mRNA. It is the first choice for most cases and generally results in a high yield of the full-length cDNA.

- Gene specific primers (GSP) have the highest specificity. But under some circumstances, GSP used for PCR reaction cannot effectively synthesize cDNA. Select Oligo dT in this case.
- Random Hexamers have the lowest specificity. All RNA, including mRNA, rRNA and tRNA can be templates of Random Hexamers. When the target area of RNA has acomplex secondary structure or is GC-rich, and Oligo dT or gene specific primers (GSP) cannot effectively synthesize cDNA, select Random Hexamers.

Choosing Primers for qPCR

- Oligo dT mixed with Random Hexamers can lead to cDNA synthesis at equivalent efficiency and helps to improve the reproducibility of quantitative results.

Protocol

◇ Guidelines for PCR reaction

1. Denaturation of RNA template*

Set up the following mixture in an RNase-free centrifuge tube.

RNase free ddH_2O	to 10 µl
Total RNA	10 pg - 5 µg
Or Poly (A)+ RNA	10 pg - 500 ng

Incubate at 65˚C for 5 minutes, place on ice rapidly and let rest for 2 minutes.

*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 omit the denaturation step.

2. Preparation of a first strand cDNA synthesis reaction mixture

Mixture of previous step	10 µl
5 x HiScript® III buffer	4 µl
dNTP Mix (10 mM each)	1 µl
HiScript® III reverse transcriptase (200 U/ul)	1 µl
RNase inhibitor (40U/µl)	1 µl
Oligo $(dT)_{20}VN$ (50 µM)	
Or Random Hexamers (100 µM)	1 µl
Or Gene Specific Primers (2 µM)	
RNase-free ddH_2O	2 µl

Mix gently with a pipette.

3. Perform the first strand cDNA synthesis reaction under the following conditions:

25˚C[a]	5 min
50˚C [b]	45 min
85˚C	5 sec

a. This step is required only when using the Random hexamers. Please omit this step when using Oligo $(dT)_{20}VN$ or Gene Specific Primer.

b. For template with complicated secondary structures or high GC content, you can raise the reaction temperature to 50˚C to increase the cDNA yield.

The product can directly be used in PCR reactions, or store at -20˚C and used within six months. It is recommended to aliquots and store at -70˚C for a long-term storage. Avoid repeated freezing and thawing.

◇ Guidelines for qPCR reaction

1. Preparation of a first strand cDNA synthesis reaction mixture

Set up the following mixture in an RNase-free centrifuge tube:

RNase free ddH_2O	to 20 μl
5x HiScript® III Buffer	4 μl
Dntp Mix (10 mM each)	1 μl
HiScript® III Reverse Transcriptase (200 U/ μl)	1 μl
RNase inhibitor (40 U/ μl)	1 μl
Oligo $(dT)_{20}VN$ (50 μM)	1 μl
Random hexamers (100 μM)	1 μl
Total RNA	10 pg - 1 μg
or Poly A+ RNA	10 pg - 100 ng

Mix gently with a pipette.

2. Perform the first 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, raise the reaction temperature to 50˚C to increase the cDNA yield.

The product can be directly used in qPCR reactions, or store at -20˚C and used within six months. It is recommended to aliquots and store at -80˚C for a long-term storage. Avoid repeated freezing and thawing.

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