HyperScript™ III First-Strand cDNA Synthesis SuperMix (with gDNA wiper)

Introduction

HyperScript™ III Reverse Transcriptase is a third-generation reverse transcriptase based on M-MLV Reverse Transcriptase that has been genetically engineered. Compared to wild-type, HyperScript™ III Reverse Transcriptase generates a reduced RNase H activity and improves thermal stability and fidelity. In addition, it has characteristics of higher cDNA synthesis yield and longer length, higher reverse transcription efficiency for high-GC content RNA. HyperScript™ III Reverse Transcriptase has an enhanced affinity for templates and is suitable for reverse transcription of small amounts of template and low-copy genes, you can use PCR to amplify cDNA up to 12.3 kb.

HyperScript™ III First-Strand cDNA Synthesis SuperMix (with gDNA wiper) is a kit based on HyperScript™ III Reverse Transcriptase, which contains all components except primers and templates required for the reaction. The kit supplies Random Primers and Oligo $(dT)_{23}VN$ primer. The inclusion of the 4× gDNA wiper in the product is intended to remove potential genomic DNA contamination before reverse transcription. Random Primers, Oligo $(dT)_{23}VN$ or gene-specific primers can be chosen as reverse transcription primers according to your needs, and the synthesized first-strand cDNA products can be used in subsequent PCR amplification, qPCR reactions, and other experiments.

Components and storage

Components	50 rxn (20 μL reaction)	100 rxn (20 μL reaction)
RNase Free ddH2O	1 mL	2×1 mL
5×HyperScript™ III SuperMix	200 μL	400 μL
4×gDNA wiper mix	190 μL	380 μL
Random Primers (50 μM)	50 μL	100 μL
Oligo $(dT)_{23}VN$ (50 μM)	50 μL	100 μL
5×HyperScript™ III control Mix	20 μL	40 μL
Store the components at -20℃ for 2 years.		

Protocol

First-strand cDNA Synthesis

1. RNA denaturation: Prepare the following mix in RNase-free PCR tubes.

Components	Volume
Rnase free ddH2O	Up to 11.25 μL
Template RNA	Total RNA: 1 pg-1 μg
Note: RNA denaturation is an optional step that helps to open secondary structures and improve reverse transcription efficiency. It should not be omitted for targets longer than 3 kb.	

Incubate at 65°C for 5 minutes, and quickly place on ice for 1 minute.

2. Removal of Genomic DNA: Prepare the following mixture, mix gently.

Components	Volume
Mixture from step 1	11.25 μL
4×gDNA wiper mix	3.75 μL

Incubate at 37°C for 2 minutes.

3. Centrifuge briefly, then prepare as the following table:

Components	Volume
Mixture from step 2	15 μL
Oligo $(dT)_{23}$VN (50 μM) or Random Primers (50 μM) or Gene Specific Primers (2 μM)	1 μL
5×HyperScript™ III SuperMix	4 μL

4. Control Reaction (Optional)

The control reaction is a reverse transcription negative control reaction without reverse transcriptase. If the reverse transcription product will be used in subsequent qPCR experiments, the control reaction can be used to verify the absence of genomic DNA contamination in the RNA sample. Prepare as the following table.

Components	Volume
Mixture from step 2	15 μL
Oligo $(dT)_{23}$VN (50 μM) or Random Primers (50 μM)	1 μL

or Gene Specific Primers (2 μM)	
5×HyperScript™ III control Mix	4 μL

5. Gently mix the above reactants, centrifuge briefly, and set up the reverse transcription program as the following table:

Temperature	Time
25°C*a	10 min
50°C*b	50 min
85°C	5 min

**Note:*

**a. This step is to be set up only when you are taking Random Primers. For Oligo(dT)23VN or Gene Specific Primers, this step is not necessary.*

**b. HyperScript™ III Reverse Transcriptase has a good amplification capability for RNA templates with complex secondary structures, generally, reactions at 50°C are recommended.*

If you can't obtain desired results at 50°C because of difficult templates or templates with a lot of secondary structures, you can increase the incubation temperature to 55°C.

The products can be used immediately in subsequent PCR or qPCR reactions, or they can be stored for short-term storage at -20°C, for long-term storage, please store at -80°C and avoid repeating freeze-thaw cycles.

[Note 1] If you need PCR to amplify some long fragments of interest (>1 kb), you may need to remove RNA that is complementary to the cDNA. You can add 2 units of E. coli RNase H (K1093, 0.4 μL) and incubate at 37°C for 20 minutes to remove RNA.

[Note 2] If the synthesized first-strand cDNA is used as a template for the PCR reaction, the amount of template added will affect the amplification efficiency of the PCR, and the addition amount should be not exceeding 1/10 of the PCR reaction volume.

Primer selection

1. If the RNA is derived from eukaryotes, it is recommended to choose Oligo(dT)23VN, which pairs with the 3' Poly(A) tail of eukaryotic mRNA for the highest yield of full-length cDNA. For prokaryotic templates, we recommend Random Primers or Gene Specific Primers.
2. If the following experiment is qPCR, a mix of Oligo(dT)23VN and Random Primers at a ratio of 1:1 can be used to improve the authenticity and reproducibility of qPCR results.
3. Random Primers have the lowest specificity and highest general applicability, and templates such as

mRNA, rRNA, tRNA, and LncRNA can all be reverse transcribed with Random Primers. In general, the recommended number of Random Primers used is 1-2 μL for cDNA shorter than 2 kb, and 0.4-1 μL for cDNA longer than 2 kb.

4. When the templates have complex secondary structures or high GC contents, and the usage of Oligo (dT)23VN or Gene Specific Primers can't lead to ideal results, Random Primers can be used.
5. Gene Specific Primer is the most specific primer. However, there are some cases where Gene Specific Primers are not effective, in this situation, reverse transcription can be re-performed with Oligo(dT)23VN or Random Primers instead.

Notes

1. The experiment is recommended to be operated on ice to avoid RNase contamination.
2. The purity of RNA will affect the amount of cDNA synthesis, and attention should be paid to prevent RNA degradation during RNA extraction.
3. If your subsequent experiment is qPCR, you may need the following products:

Catalog number	Product name
K1070	HotStart™ 2X SYBR Green qPCR Master Mix
K1170	HotStart™ Universal 2X SYBR Green qPCR Master Mix
K1171	HotStart™ 2X FAST SYBR Green qPCR Master Mix
K1172	HotStart™ Universal 2X FAST SYBR Green qPCR Master Mix
K1541	HotStart™ 2X Probe qPCR Master Mix
K1542	HotStart™ Universal 2X Probe qPCR Master Mix

4. This product is for scientific research purposes only.

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