HyperScript™ III RT SuperMix for qPCR

Introduction

HyperScript™ III Reverse Transcriptase is a third-generation reverse transcriptase based on M-MLV Reverse Transcriptase that has been genetically engineered. 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 as well as low-copy genes, you can use PCR to amplify cDNA up to 12.3 kb.

The HyperScript™ III RT SuperMix for qPCR is designed for two-step qRT-PCR assays, 5 × HyperScript™ III SuperMix contains all components for reverse transcription except template RNA. The maximum volume of the RNA template can be up to 80% of the total volume, making the kit ideal for reverse transcription of low concentrations RNA templates. The 5X HyperScript™ III SuperMix doesn't freeze at -20°C and is easy to use.

This product has been optimized for qPCR, especially the ratio of Oligo $(dT)_{23}$VN primers /Random primers, the cDNA synthesis can be initiated from all regions of the RNA transcript with the same efficiency, ensuring maximum authenticity and reproducibility of qPCR results. The reverse transcription products can be employed in SYBR Green or probe-based qPCR, you can choose appropriate reagents for gene expression analysis according to your purpose.

Components and storage

Components	50 rxn (20 μL reaction)	100 rxn (20 μL reaction)
RNase Free ddH_2O	1 mL	2 × 1 mL
5 X HyperScript™ III RT SuperMix	200 μL	400 μL
5 X HyperScript™ III No RT control Mix	20 μL	40 μL
Store the components at -20℃ for 2 years.		

Protocol

1. First-strand cDNA synthesis

Prepare the following mixture in RNase-free PCR tubes:

Components	Volume
RNase Free ddH2O	Up to 20 μL
5 X HyperScript™ III RT SuperMix	4 μL
Template RNA	Total RNA: 1 pg - 1 μg

2. No RT Control reaction (optional)

No RT Control reaction refers to a reverse transcription negative control reaction without reverse transcriptase to verify the absence of genomic DNA contamination in the RNA sample. Prepare the following mixture in RNase-free tubes:

Components	Volume
RNase Free ddH2O	Up to 20 μL
5 X HyperScript™ III No RT control Mix	4 μL
Template RNA	Total RNA: 1 pg - 1 μg

3. Reverse transcription

Gently mix, then centrifuge briefly, and set up the reverse transcription program as the following table:

Temperature	Time
25°C	10 min
50°C	15 min
85°C	5 min
Note: HyperScript™ III Reverse Transcriptase has a good amplification capability for RNA templates with complex secondary structures. Typically, it is recommended to perform the experiments at 50 ℃. If you can't generate desired results at 50 ℃, you can increase the temperature to 55 ℃.	

The products can be used immediately in qPCR reactions or stored for a short time at -20°C, for long-term storage, it is recommended to store at -80°C and avoid repeating freeze-thaw cycles. Storage at -20°C should not exceed 1 week.

Notes

1. The experiment should be operated on ice to avoid RNase contamination during the process.
2. The purity of RNA will affect the yield of cDNA synthesis, and attention should be paid to prevent RNA degradation during RNA extraction.
3. The 5x HyperScript™ III RT SuperMix and 5× HyperScript™ III No RT Control Mix contain a high concentration of glycerol; you may centrifuge briefly before use and pipette correctly.
4. For a 20 μL reverse transcription reaction, it is recommended to add no more than 1 μg of total RNA, and if the expression of the target gene is extremely low, a maximum of 5 μg total RNA can be used. Excessive addition of RNA may lead to the result of exceeding the linear range of subsequent quantitative PCR.
5. If the volume of the RNA template is large (e.g., more than 2 μL), make sure that the RNA is dissolved in water, not in TE, as EDTA in TE can inhibit the reverse transcription reaction.
6. If a difference in CT values less than 5 shows between HyperScript™ III No RT Control and the positive group in the qPCR experiment, it is possible that the RNA template has been contaminated by genomic DNA.
7. The cDNA product of this kit is only suitable for qPCR reactions and is not applicable for PCR amplification of long fragments. If need, the HyperScript™ III First-Strand cDNA Synthesis Kit (Cat. No. K1581) is recommended.
8. 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

9. This product is for scientific research purposes only.

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