HyperScript™ IV RT SuperMix for qPCR

Description

HyperScript™ IV Reverse Transcriptase is a genetically engineered fourth-generation reverse transcriptase based on M-MLV and provides superior robustness and reliability in RT reactions. The enzyme has significant improvements in inhibitor resistance, processivity, and reaction speed while retaining all the advantages of HyperScript™ III Reverse Transcriptase, including thermostability, highly efficient full-length cDNA synthesis, and reduced RNase H activity. The product can still provide reliable, consistent, and rapid cDNA synthesis in the presence of inhibitors (residues from RNA extraction).

The HyperScript™ IV RT SuperMix for qPCR is designed for two-step RT-qPCR assays, 5 × HyperScript™ IV RT 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.

This product has been optimized for qPCR, especially the ratio of Oligo (dT)23VN primer /Random primer, 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 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 rxns (20 μL reaction)	100 rxns (20 μL reaction)
RNase Free ddH2O	1 mL	2 X 1 mL
5 X HyperScript™ IV RT SuperMix	200 μL	400 μL
5 X HyperScript™ IV 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
5 X HyperScript™ IV RT SuperMix	4 µL
Template RNA	Total RNA: 1 pg – 2.5 µg
RNase Free ddH2O	Up to 20 µL

2. No RT Control reaction (optional)

No RT Control refers to a reverse transcription negative control 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
5 X HyperScript™ IV No RT control Mix	4 µL
Template RNA	Total RNA: 1 pg – 2.5 µg
RNase Free ddH2O	Up to 20 µL

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	10 min
85°C	5 min

The products can be used immediately in qPCR reactions, or they can be stored for a short term at -20°C. For long term storage, please 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.
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. 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.
4. If a difference in CT values less than 5 shows between HyperScript™ IV No RT Control and the positive

group in the qPCR experiment, it is possible that the RNA template has been contaminated by genomic DNA.

5. The cDNA product is only suitable for qPCR reactions and is not applicable for PCR amplification of long fragments. If need, the HyperScript™ IV First-Strand cDNA Synthesis Kit (Cat. No. K1586) is recommended.

6. For the subsequent 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

7. This product is for scientific research purposes only.

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