

HyperScript™ IV RT SuperMix for qPCR (with gDNA wiper)

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 (with gDNA wiper) is suitable for two-step RT-qPCR assays, 5 × HyperScript™ IV RT SuperMix contains all components for reverse transcription except the template. The inclusion of the 4× gDNA wiper in the product is intended to remove potential genomic DNA contamination before reverse transcription. This kit is ideal for reverse transcription of low-concentration 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 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 rxns (20 µL reaction)	100 rxns (20 µL reaction)
RNase Free ddH ₂ O	1 mL	2×1 mL
4×gDNA wiper mix	200 µL	400 µL
5×HyperScript™ IV RT SuperMix	200 µL	400 µL
5×HyperScript™ IV No RT control Mix	20 µL	40 µL

Store the components at -20°C for 2 years.

Protocol

1. Removal of Genomic DNA

Prepare the following mixture in RNase-free PCR tubes:

Components	Volume
RNase Free ddH ₂ O	Up to 16 uL
4×gDNA wiper mix	4 uL
Template RNA	Total RNA: 1 pg -2.5 µg

Mix gently with a pipette and incubate:

Temperature	Time
37°C	2 min

2. First-strand cDNA synthesis

Prepare as the following table:

Components	Volume
Mixture from step 1	16 µL
5×HyperScript™ IV RT SuperMix	4 µL

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 as the following table:

Components	Volume
Mixture from step 1	16 µL
5×HyperScript™ IV No RT control Mix	4 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
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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. 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)/HyperScript™ IV First-Strand cDNA Synthesis SuperMix (Cat. No. K1587)/HyperScript™ IV First-Strand cDNA Synthesis SuperMix (with gDNA wiper) (Cat. No. K1589) 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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