HiScript® III All-in-one RT SuperMix Perfect for qPCR

Cat. No. NB-54-0222-01

Product Description

HiScript III All-in-one RT SuperMix Perfect for qPCR is an upgraded version of HiScript III RT SuperMix for qPCR(+gDNA wiper). Genomic DNA elimination and reverse transcription can be simultaneously completed, which is convenient. And it can greatly reduce contamination and RNA degradation. This kit contains HiScript III Reverse Transcriptase, heat-labile DNase and an optimized buffer system. Among them, heat-labile DNase takes effect quickly. It has high efficiency, and can be easily inactivated. The obtained cDNA can be stored stably for a long time. It is compatible with dye-based and probe-based qPCR, enabling high-performance gene expression analysis.

Components

Components	NB-54-0222-01 100 rxns (20 μl/rxn)
RNase-free ddH_2O	2 × 1 ml
Enzyme Mix*	100 μl
5 × All-in-one qRT SuperMix*	400 μl
NO RT Control Mix*	20 μl

* Please put Enzyme Mix, 5 × All-in-one qRT SuperMix and No RT Control Mix on ice, owing to the DNase is heat-labile.

Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

Applications

It is suitable for reverse transcription of animal, plant and microbial RNA. The products are compatible with dye-based and probe-based qPCR.

Self-prepared Materials

RNase-free 1.5 ml centrifuge tubes, 0.2 ml PCR tubes, pipette tips, pipettes, ice box;
PCR instrument;
Dye-based qPCR reagent (e.g., ChamQ Universal SYBR qPCR Master Mix, NB-54-0173) or probe-based qPCR reagent (e.g., AceQ Universal U^+ Probe Master Mix V2, NB-54-0172).

Notes

1. After reaction system preparation, mix thoroughly by pipetting up and down 8 - 10 times.
2. Please put Enzyme Mix, 5 × All-in-one qRT SuperMix and No RT Control Mix on ice, owing to the DNase is heat-labile.
3. Enzyme Mix, 5 × All-in-one qRT SuperMix and No RT Control Mix contain high concentration of glycerol, please centrifuge briefly before use and pipette up and down to mix thoroughly.
4. It is recommended to add no more than 1 μg Total RNA to the 20 μl reverse transcription reaction system. If the expression level of the target gene is very low, add up to 5 μg Total RNA. Otherwise the amount of RNA added is too high, which may exceed the linear range of subsequent qPCR.
5. If the cDNA product stock solution is directly used as the template of the qPCR, it is recommended that the volume of the cDNA product does not exceed 1/10 of the reaction system.
6. The reverse transcription products can be used immediately in the subsequent qPCR, or stored at -30 ~ -15℃, and used within half a year. For long term storage, store at -85 ~ -65℃ after aliquoting. Avoid repeated freezing and thawing.

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Experiment Process

1. Reaction system preparation

Prepare the reaction mixture in RNase-free tubes as follows:

RNase-free ddH_2O	to 20 μl	☐
5 × All-in-one qRT SuperMix	4 μl	■
Enzyme Mix	1 μl	■
Template RNA	Total RNA：1 pg - 1 μg	

Mix thoroughly by pipetting up and down 8 - 10 times*. Centrifuge briefly to collect the solution at the bottom of the tubes.

* Be sure to mix thoroughly, otherwise it will affect the stability of the experimental data.

No RT Control reaction (optional)

No RT Control refers to the negative control reaction of reverse transcription without reverse transcriptase, which is used to check whether there is residual genomic DNA in the RNA template.

Prepare the reaction mixture in RNase-free tubes as follows:

RNase-free ddH_2O	to 20 μl	☐
5 × All-in-one qRT SuperMix	4 μl	■
No RT Control Mix	1 μl	■
Template RNA	Total RNA：1 pg - 1 μg	

Mix thoroughly by pipetting up and down 8 - 10 times*. Centrifuge briefly to collect the solution at the bottom of the tubes.

* Be sure to mix thoroughly, otherwise it will affect the stability of the experimental data.

2. PCR program

50˚C	15 min
85˚C	5 sec

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