

HyperScript™ RT SuperMix for qPCR Protocol

Introduction

HyperScript™ RT SuperMix for qPCR is the premixed solution for reverse transcription reaction, based on HyperScript™ Reverse Transcriptase (Cat. No. K1071). HyperScript™ Reverse Transcriptase is a new enzyme obtained through genetic engineering based on M-MLV (RNase H⁻) Reverse Transcriptase. In comparison, HyperScript™ Reverse Transcriptase reduces RNase H activity and increases thermal stability. HyperScript™ Reverse Transcriptase can withstand higher reaction temperatures and is suitable for reverse transcription of RNA templates with complex secondary structures.

HyperScript™ RT SuperMix for qPCR is suitable for two-step qRT-PCR detection, 5× SuperMix contains all the components required for reverse transcription, and the reaction can be carried out quickly by only adding template RNA and RNase Free ddH₂O. The volume of the RNA template can be added up to 80% of the total volume, which is very suitable for the reverse transcription reaction of the low concentration RNA template. 5× SuperMix will not freeze at -20°C, easy to use.

This product is specially optimized for qPCR. The proportionally optimized Oligo (dT)₂₃VN primer mix/random primers enables cDNA synthesis to start from various regions of the RNA transcript with the same reverse transcription efficiency, maximizing the authenticity and repeatability of the qPCR results. Reverse transcription products are suitable for qPCR by SYBR Green and probe methods. The corresponding reagents can be selected for high performance gene expression analysis according to the experimental 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
5× RT SuperMix	200 µL	400 µL
5× No RT control Mix	20 µL	40 µL

Store the components at -20°C.

Protocol

1. Preparation of first chain cDNA synthetic reaction in RNase free PCR tube.

Components	volume
RNase Free ddH ₂ O	Up to 20 µL
5× SuperMix	4 µL
Templet RNA	Total RNA: 1 pg - 1 µg

No RT Control reactions (optional)

No RT Control refers to the negative reverse transcription reaction without HyperScript™ Reverse Transcriptase, which is used to test whether there are genomic DNA residues in the RNA template.

prepare the following mixed solution in RNase free PCR tube.

Components	volume
RNase Free ddH ₂ O	Up to 20 µL
5 × No RT control Mix	4 µL
Templet RNA	Total RNA: 1 pg - 1 µg

2. Reverse transcription reaction

Mix the above reactants gently, centrifuge instantly and set the reverse transcription procedure according to the following table:

temperature	time
25°C	2 mins
42-50°C	15 mins
95°C	1 min

Note: HyperScript™ Reverse Transcriptase still has good amplification ability for RNA templates with complex secondary structures, it is usually recommended to react at 42°C. it can also be reacted in 45-50°C in order to reduce nonspecific amplification and increase yield.

The obtained reverse transcript products can be used immediately for qPCR reactions and can also be stored in -20°C for short-term preservation and -80°C for long-term preservation, avoiding repeated freezing and thawing.

■ Matters need attention

1. Experiments were operated on ice to avoid RNase contamination during the process.
2. The purity of RNA may affect the amount of cDNA synthesis, so the RNA extraction process should pay attention to prevent RNA degradation.
3. The 5×SuperMix and 5×SuperMixNo RT Control Mix contain high concentration of glycerin, before using please briefly centrifuge collection to the bottom of the reaction tube and mix it gently and thoroughly with the liquid remover.
4. For the 20µl reverse transcription reaction system, it is recommended to add no more than 1µg Total RNA, if the target gene expression is very low, add up to 5µg total RNA, otherwise the amount of addition is too high, which may be beyond the linear range of subsequent qPCR.

5. Make sure RNA is soluble in water rather than in TE if the volume of added template is too much (more than 2μl), as the EDTA in the TE will inhibit the reverse transcription reaction.
6. The RNA template may be contaminated by genomic DNA if the CT value difference between No RT Control and experimental group is less than 5.
7. The cDNA products are only suitable for qPCR reactions and not for long fragment PCR amplification of downstream experiments such as cloning. First-Strand cDNA Synthesis Kit (Cat. No. K1071) is recommended if required.



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