HyperScript™ First-Strand cDNA Synthesis SuperMix (with gDNA wiper)

Introduction

HyperScript™ First-Strand cDNA Synthesis SuperMix (with gDNA wiper) is based on HyperScript™ Reverse Transcriptase (Cat. No. K1071) to synthesize first-strand cDNA from Total RNA or Poly(A)$^+$ RNA. HyperScript™ Reverse Transcriptase is a new enzyme obtained through genetic engineering based on M-MLV (RNase H$^-$) Reverse Transcriptase. In Transcriptase can withstand higher reaction temperatures and is suitable for reverse transcription of RNA templates with complex secondary structures. In addition, HyperScript™ Reverse Transcriptase enhances the affinity to the template, suitable for the reverse transcription of a small number of templates and low copy genes and can generate cDNA up to 12.3 kb.

HyperScript™ First-Strand cDNA Synthesis SuperMix (with gDNA wiper) contains all components except primers required for the synthesis of the first strand cDNA and provides two cDNA synthesis primers, Random Primers and Oligo $(dT)_{23}VN$. The 4× gDNA wiper included in the product quickly and thoroughly removes potential genomic interference from the system before reversing transcription. You can choose Random Primers, Oligo $(dT)_{23}VN$ or gene-specific primers as reverse transcription primers according to the needs of the experiment. The synthesized first-strand cDNA products can be used in subsequent experiments such as PCR amplification and qPCR reaction.

Components and Storage

Components	50 rxns (20 μL reaction)	100 rxns (20 μL reaction)
RNase Free ddH_2O	1 mL	2×1 mL
5×SuperMix	200 μL	400 μL
4×gDNA wiper mix	190 μL	380 μL
Random Primers (50 μM)	50 μL	100 μL
Oligo $(dT)_{23}VN$ (50 μM)	50 μL	100 μL
5×control Mix	20 μL	40 μL
Store the components at -20°C.		

First-strand cDNA synthesis

1. Remove genomic DNA：prepare the following mixed solution in RNase free PCR tube.

Component	Volume
RNase Free ddH_2O	Up to 15 μL

4×gDNA wiper mix	3.75 uL
Template RNA	Total RNA: 1 pg – 5 μg poly(A) mRNA: 0.1 pg – 500 ng

Gently mix the above reactions: Incubate at 37°C for 2-5min; 55°C for 5min.

2. RNA denaturation*, prepare the following mixed solution in RNase free PCR tube.

Component	Volume
Oligo (dT) $_{23}$VN (50 μM) or Random Primers (50 μM) or Gene Specific Primers (2 μM)	1 μL
Reaction solution of Step 1	15 μL

***Note: The RNA denaturation step is optional. RNA denaturation helps to open the secondary structure and improve the efficiency of reverse transcription. For cDNA fragments longer than 3 kb, do not omit this step.**

3. Heat mixture at 65°C for 5 min, and then quickly cool on ice for 2 min.

4. Centrifuge immediately and prepare a reverse transcription reaction system.

Component	Volume
Reaction solution of Step 1	16 μL
5x SuperMix	4 μL

No RT Control reactions (optional)

Component	Volume
Reaction solution of Step 1	16 μL
5x control Mix	4 μL

5. Gently mix the above reactions. Centrifuge immediately and set the reverse transcription program according to the following table.

Temperature	Time
25°C [*a]	2 min
42-50°C [*b]	50 min
75°C	15 min

***Note: a. If using Random Primers, you need to set this step; if using Oligo (dT)$_{23}$ VN or Gene Specific Primers, you can omit this step.**

b. HyperScript™ Reverse Transcriptase still has good amplification ability for RNA templates with complex secondary structures, so it is generally recommended to perform the reaction at 42°C. When specific downstream primers are used for reverse transcription, non-specific products may be amplified due to mismatches. In this case, the reaction can be performed at 45-50°C to reduce non-specific amplification.

6. The obtained reverse transcription products can be used immediately for subsequent PCR or qPCR reactions, and can also be stored at -20°C for short-term storage and long-term storage at -80°C to avoid repeated

freezing and thawing.*

***Note: If the first-strand cDNA is used as a template for the PCR reaction, the amount of template added will affect the amplification efficiency of PCR. The amount of template added is less than 1/10 of the amount of the PCR reaction solution. In the RT-PCR reaction, if there is non-specific amplification or no amplification products, the first strand cDNA synthesis reaction solution can be treated with RNase H (for example, 1 μL RNase H is added to the synthesis reaction solution and incubated at 37°C for 20 minutes).**

Primer selection

1. If it is a eukaryotic template, it is recommended to choose Oligo $(dT)_{23}VN$. This primer can be paired with the 3' Poly(A) tail of the eukaryotic mRNA to obtain the highest yield of full-length cDNA. For prokaryotic templates, we recommend Random Primers or Gene Specific Primers.
2. When the subsequent experiments are qPCR, you can mix Oligo $(dT)_{23}VN$ with Random Primers 1: 1 to improve the authenticity and repeatability of qPCR results.
3. Random Primers have the lowest specificity and wide applicability. All templates such as mRNA, rRNA, tRNA and LncRNA can be reverse transcribed using Random Primers. Generally, the amount of Random Primers used for cDNA synthesis below 2 kb is 1-2 μL; the amount of Random Primers used during cDNA synthesis above 2 kb is 0.4-1 μL.
4. When the template has a complex secondary structure or a high GC content, Oligo $(dT)_{23}VN$ or Gene Specific Primers cannot effectively guide cDNA synthesis, Random Primers can be used as primers.
5. Gene Specific Primers are the most specific. However, in some cases, Gene Specific Primers cannot effectively guide first-strand cDNA synthesis. Reverse transcription can be performed with Oligo $(dT)_{23}VN$ or Random Primers instead.

Note

1. Experiments are performed on ice; RNase contamination should be avoided during the process.
2. The purity of RNA will affect the amount of cDNA synthesis. The RNA extraction process should pay attention to prevent RNA degradation.

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