First-Strand cDNA Synthesis SuperMix (with gDNA wiper)

Introduction

First-Strand cDNA Synthesis SuperMix (with gDNA wiper) is based on Reverse Transcriptase (Cat. No. K1071) to synthesize first-strand cDNA from Total RNA or Poly(A)$^+$ RNA. Reverse Transcriptase is a new enzyme obtained through genetic engineering based on M-MLV (RNase H$^-$) Reverse Transcriptase. In comparison, Reverse Transcriptase reduces RNase H activity and increases thermal stability. Reverse Transcriptase can withstand higher reaction temperatures and is suitable for reverse transcription of RNA templates with complex secondary structures. In addition, 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.

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 \ Size	50 rxns (20 μL reaction)	100 rxns (20 μL reaction)	Storage
RNase Free ddH_2O	1 mL	2×1 mL	-20℃
5 × SuperMix	200 μL	400 μL	-20℃
4 × gDNA wiper mix	190 μL	380 μL	-20℃
Random Primers (50 μM)	50 μL	100 μL	-20℃
Oligo (dT) $_{23}$VN (50 μM)	50 μL	100 μL	-20℃
5 × control Mix	20 μL	40 μL	-20℃
Shipping: Dry Ice	**Shelf life:** 12 months		

First-strand cDNA synthesis

1. Remove genomic DNA：prepare the reactions in RNase free PCR tube.

Components	Volume
RNase Free ddH_2O	Up to 15 μL
4 × gDNA wiper mix	3.75 μL
Template RNA	Total RNA 1 pg - 1 μg or poly(A) mRNA 0.1 pg – 100 ng for Random Primers Total RNA 1 pg - 5 μg or poly(A) mRNA 0.1 pg – 500 ng for oligo$(dT)_{23}$ VN or Gene Specific Primers

Gently mix.

Temperature	Time
37℃	2 min
55℃	5 min

2. RNA denaturation:

Components	Volume
Oligo $(dT)_{23}$VN (50 μM) or Random Primers (50 μM) or Gene Specific Primers (2 μM)	1 μL
Mixture from Step 1	15 μL

Incubate the mixture at 65℃ for 5 min, and then quickly cool on ice for 2 min.

Notes： a. The RNA denaturation step is optional. RNA denaturation helps to open the secondary structure and improve the efficiency of reverse transcription.

b. Due to the Mg^{2+} in the 4 × gDNA wiper mix, RNA will be fragmented into short segments after treatment with the 4 × gDNA wiper mix. In this step, it is more advisable to select Random primers. If choosing Oligo$(dT)_{23}$ VN or Gene Specific Primers, you can't generate longer fragments (>750 bp).

3. Centrifuge immediately and prepare the reverse transcription reaction system.

Components	Volume
Mixture from Step 2	16 μL
5 × SuperMix	4 μL

Control reactions (optional):

The Control reaction refers to the reverse transcription reaction without the addition of reverse transcriptase. If the reverse transcription products will be used in the subsequent qPCR experiments, the Control reaction can be used to identify whether there is genomic DNA residue in the RNA template.

Prepare as the following table：

Components	Volume
Mixture from Step 2	16 μL
5 × control Mix	4 μL

4. Gently mix. Then Centrifuge immediately and set the reverse transcription program according to the following table.

Temperature	Time
25℃ *a	2 min
42-50℃ *b	50 min
75℃	15 min

Notes: 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. Generally, it is recommended to perform the reaction at 42℃. When specific primers are use in the subsequent PCR or qPCR experiments, non-specific products may be amplified due to mismatches. In this case, the reaction can be performed at 45-50℃ to reduce non-specific amplification.

5. The obtained reverse transcription products can be used immediately for subsequent PCR or qPCR reactions. Or you can store at -20℃ for short-term storage and -80℃ for long-term storage. Take care to avoid repeated freeze/thaw cycles.

Notes: a. The obtained reverse transcription product can only be used for subsequent PCR amplification if its length is less than 750 bp.

b. If the first-strand cDNA is used as a template in the PCR reaction, the amount of template added will affect the amplification efficiency of PCR. It is recommended that the volume of template added not exceed 1/10 of the total volume. In the RT-PCR reaction, if there is non-specific amplification or no amplification products, the reverse transcription products can be treated with 2 units RNase H. You can add 0.4 μL RNase H (K1093) to the reverse transcription products and incubated at 37℃ for 20 minutes.

Primer selection

1. When the subsequent experiment is qPCR, you can mix Oligo $(dT)_{23}VN$ with Random Primers in the ratio of 1:1 to improve the authenticity and repeatability of qPCR results.
2. 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.
3. 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.
4. 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 should be performed on ice; RNase contamination should be avoided during the process.
2. The purity of RNA will affect the yield of cDNA. In the RNA extraction process, you should pay attention to prevent RNA degradation.

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