First Strand cDNA Synthesis Kit (+gDNA wiper)

（M002）

Product Description

First Strand cDNA Synthesis Kit (+gDNA wiper) contains a new-generation reverse transcriptase and an optimized buffer specifically formulated for reverse transcription, which further improves first-strand synthesis efficiency.

The 5× gDNA wiper Mix included in the kit can rapidly remove genomic DNA contamination at 42 °C for 2 min, ensuring more reliable subsequent results and simplifying qPCR primer design without the need for intron-spanning primers.

The kit provides individual reverse transcription primers: Oligo$(dT)_{18}$VN and Random Primers, allowing users to flexibly choose primers for downstream experiments.

This kit can synthesize both full-length cDNA for cloning and other downstream applications, as well as highly uniform cDNA for qPCR quantification. It is suitable for reverse transcription of RNA from animals, plants, and microorganisms.

Product Components

Cat. No.	Product Name	Volume（500 rxns/） M002
RM002-A	5×gDNA wiper Mix	1.0 mL
RM002-B	4×RT Mix	2.5 mL
RM002-C	RT III Enzyme Mix	1.0 mL
RM002-D	Oligo $(dT)_{18}$VN	500 μL
RM002-E	Random Primers	500 μL
RM002-F	Nuclease-free Water	2.0 mL

Storage Conditions

Store at -30 ~ -15 °C. Transport at ≤ 0 °C. Valid for 12 months.

Precautions

1. Wear disposable gloves and a mask during the experiment and follow standard operating procedures.
2. This product is a reverse transcription reagent for cDNA synthesis from RNA. Strictly prevent RNase contamination during RNA extraction and reverse transcription. Wear gloves and a mask, and use RNase-free laboratory consumables.
3. For subsequent PCR experiments: If the template is derived from eukaryotes, **Oligo$(dT)_{18}$VN** is generally the first choice. It anneals to the 3' poly(A) tail of eukaryotic mRNA and yields the highest amount of full-length cDNA. **Gene-Specific Primers (GSP)** provide the highest specificity. However, in some cases, GSP for PCR may not efficiently prime first-strand cDNA

synthesis. In such instances, reverse transcription can be repeated using Oligo(dT)$_{18}$VN or Random Primers. Random Primers have the lowest specificity and can use all RNA species as templates, including mRNA, rRNA, and tRNA. Random Primers are recommended when the target region has a complex secondary structure, high GC content, or when the template is derived from prokaryotes and cannot be efficiently primed by Oligo(dT)$_{18}$VN or GSP.

4. For subsequent qPCR experiments: Using a mixture of Oligo(dT)$_{18}$VN and Random Primers at the recommended ratio ensures uniform cDNA synthesis efficiency across different regions of mRNA, which helps improve the accuracy and reproducibility of quantitative results.Reverse transcription can be performed directly without a genomic DNA removal step; the remaining volume can be adjusted with Nuclease-free Water.

Protocol

For subsequent PCR experiments：

1. RNA Template Denaturation

Prepare the following mixture in an RNase-free centrifuge tube：

Components	Volumes
Total RNA	10 pg - 5 μg
or Poly A+ RNA	10 pg - 500 ng
Nuclease-free Water	To 20 μL

Heat at 65 °C for 5 min, chill immediately on ice, and keep on ice for 2 min.

Notes：

(1) RNA template denaturation helps unwind secondary structures and greatly improves the yield of first-strand cDNA. Do not omit this denaturation step for cDNA fragments longer than 3 kb.

(2) Recommended amount: 1 μg of **Total RNA or Poly A+ RNA**.

2. Genomic DNA Removal

Components	Volumes
The mixture from the previous step	8 μL
5×gDNA wiper Mix	2 μL

Mix gently by pipetting. Incubate at 42 °C for 2 min.

3. Prepare the first-strand cDNA synthesis reaction mixture：

Components	Volumes
The mixture from the previous step	10 μL
4×RT Mix	5 μL
RT III Enzyme Mix	2 μL

Oligo $(dT)_{18}$VN or Random Primers	1 μL
Nuclease-free Water	2 μL

Notes： This product is also suitable for reverse transcription primed by **gene-specific primers (GSP)**. To avoid potential effects of gDNA wiper on GSP, add **2 pmol of gene-specific primer** to the reaction mixture in this step.

4. Reaction Conditions for First-Strand cDNA Synthesis：

Temperature	Time
25℃*1	5 min
50℃*2	45 min
85℃	5 sec

Notes：

*1： This step is **only required when using Random Primers**; omit it when using Oligo$(dT)_{18}$VN or GSP.

*2： If the template has complex secondary structure or high GC-rich regions, the reaction temperature can be increased to 60 °C to improve yield.

5. The product can be used immediately for PCR, or stored at -20 °C and used within six months.

For long-term storage, aliquot and store at -70 °C. cDNA should be avoided from repeated freeze-thaw cycles.

For subsequent qPCR experiments：

1. **Genomic DNA Removal**

Components	Volumes
Total RNA	10 pg - 1 μg
or Poly A+ RNA	10 pg - 100 ng
5×gDNA wiper Mix	2 μL
Nuclease-free Water	To 10 μL

Mix gently by pipetting. Incubate at 42 °C for 2 min.

2. **Prepare the first-strand cDNA synthesis reaction mixture：**

Components	Volumes
The mixture from the previous step	10 μL
4×RT Mix	5 μL
RT III Enzyme Mix	2 μL
Oligo $(dT)_{18}$VN	1 μL
Random Primers	1 μL
Nuclease-free Water	1 μL

Notes：This product is also suitable for reverse transcription primed by gene-specific primers (GSP). To avoid potential interference

of gDNA wiper with GSP, add 2 pmol of gene-specific primer to the reaction system at this step.

3. **Reaction conditions for first-strand cDNA synthesis：**

Temperature	Time
50℃[*1]	15 min
85℃	5 sec

*1：If the template contains complex secondary structures or high-GC regions, the reaction temperature can be increased to 60 °C to improve the yield.

4. **The product can be used immediately for qPCR, or stored at -20 °C and used within six months. For long-term storage, aliquot and store at -70 °C. Avoid repeated freeze-thaw cycles of cDNA.**