Instruction for Use

Evo Super M-MLV Plus 1st Strand cDNA Synthesis Mix

AG11623
Version.V1E1

Research Use Only
Not For Diagnosis Procedures

1. Description

This product is a kit designed for first-strand cDNA synthesis starting from total RNA or poly(A)+ RNA. The required components for first-strand cDNA synthesis, including Evo Super M-MLV Plus reverse transcriptase, RNase inhibitor, oligo(dT) primer, dNTP mixture, and reaction buffer, are pre-mixed in a Premix format, allowing users to initiate the reaction simply by adding the RNA template and nuclease-free water. By incorporating auxiliary proteins and an optimized buffer system, this product minimizes the need for RNA denaturation prior to reverse transcription, resulting in a more convenient and streamlined workflow.

The kit features high synthesis efficiency, rapid reaction speed, high sensitivity, excellent thermal stability, and strong inhibitor tolerance, enabling efficient first-strand cDNA synthesis over a temperature range of 42°C to 60°C. In addition, high-temperature reverse transcription improves efficiency and specificity when gene-specific primers are used for first-strand cDNA synthesis. The synthesized first-strand cDNA is suitable for second-strand cDNA synthesis, hybridization, PCR amplification, and full-length cDNA library construction. The kit also includes Random Hexamer primers, enabling cDNA synthesis from RNA lacking poly(A)+ tails, and is compatible with real-time PCR (qPCR) applications for gene expression analysis.

2. Kit Information

Kit Name	Cat. No	Specification
Evo Super M-MLV Plus 1st Strand cDNA Synthesis Mix	AG 11623	50 rxns

3. Transportation and Storage

Storage	Store at –20°C
Transportation	Transport at –20°C Dry Ice or Blue Ice Condition

4. Kit Components

Kit Components	Volume
4X *Evo Super M-MLV* Plus cDNA Synthesis Mix*1	250 μl
Random 6 mers Primer (100uM)*2	100 μl
RNase Free Water	1 ml x 2 Pcs

*1: Contains Evo Super M-MLV reverse transcriptase, RNase inhibitor, Oligo dT (18T) primer, dNTP mixture, and reaction buffer.

*2：This component should be added to the reaction mixture when synthesizing cDNA from RNA lacking poly(A)+ tails, for cDNA preparation for real-time PCR, or for uniform cDNA synthesis across the entire RNA transcript.

5. Precautions and Preparation

1) The 4× Evo Super M-MLV Plus cDNA Synthesis Mix contains a relatively high glycerol concentration. Before use, briefly centrifuge to collect all liquid at the bottom of the tube to minimize sample loss. Gently mix by pipetting and avoid introducing bubbles. After use, it is recommended to promptly store the reagent at –20°C.
2) During operation, take precautions to prevent RNase contamination from the working environment and handling procedures.
3) The reaction mixture should be prepared on ice, and the assembled reaction should be immediately placed into the PCR instrument for incubation.
4) Reagents & Consumables: Primers, RNase-free water, RNase-free 1.5 mL microcentrifuge tubes, RNase-free PCR tubes, and RNase-free pipette tips.
5) Instruments: PCR thermal cycler, pipettes, vortex mixer, mini benchtop centrifuge, electrophoresis system, and gel imaging system.

6. Protocol

6.1 Reverse Transcription for downstream PCR analysis

First Strand cDNA Synthesis

1) Prepare the reaction mix as below on ice.

Components	Input Volume
4X Evo Super M-MLV Plus cDNA Synthesis Mix	5 μl
Random 6 mers Primer (100μM) [*1,*2]	0~2 μl
Template RNA	Total RNA ≤5μg / Poly(A)+RNA ≤1μg
RNase free water	Up to 20 μl [*3]

*1: If the RNA template contains a poly(A)+ tail, the Random 6 mers Primer may be omitted.
When Random 6 mers Primer is required for reverse transcription:
For synthesis of cDNA fragments ≤ 2 kb, 1 μL is recommended; the volume may be adjusted within 1–2 μL as needed.
For synthesis of cDNA fragments > 2 kb, the recommended amount of Random 6 mers Primer is 0–1 μL.
*2: A Gene-Specific Primer (GSP) may also be used; in this case, the final concentration in the reaction system should be 0.1 μM.

2) Run the cDNA Synthesis Reaction Setup as below.

Temperature	Time
30°C	5 min[*1]
55°C[*2]	15 min[*3]
85°C[*4]	5 min
4°C	-

*1: If Random 6 mers Primer is not used for reverse transcription, this step may be omitted.
When Random 6 mers Primer is used, a 30 °C incubation for 5 min prior to the reaction is recommended to allow efficient primer annealing to the template and to improve reverse transcription efficiency. The annealing time may be adjusted within 0-10 min according to experimental needs. If 30 °C for 5 min does not yield satisfactory results, the annealing time may be extended to 10 min.
*2: Evo Super M-MLV Plus exhibits excellent extension performance even with templates containing complex secondary structures. A reverse transcription temperature of 55 °C is generally sufficient to achieve optimal results, but the temperature may be adjusted within the range of 50-60 °C as required. For longer cDNA targets or more complex templates, increasing the temperature to 55-60 °C is recommended. When using Gene-Specific Primers, the reverse transcription temperature may also be adjusted within 50-60 °C.
*3: Under most conditions, a 15 min reverse transcription reaction yields satisfactory results. For cDNA lengths < 4 kb, the reaction time may be adjusted within 5-15 min as needed. For longer cDNA targets or more complex templates, the reverse transcription time may be extended to 15-30 min.
*4: For long-fragment cDNA amplification, a 70 °C incubation for 15 min is recommended to ensure complete inactivation of the reverse transcriptase and to preserve the integrity of the first-strand cDNA.

3) The cDNA solution obtained above can be used directly for subsequent PCR amplification. The volume of cDNA added should not exceed 1/10 of the total PCR reaction volume.

6.2 Reverse Transcription for downstream Real Time PCR analysis

First Strand cDNA Synthesis

1) Prepare the reaction mix as below on ice.

Components	Input Volume

4X Evo Super M-MLV Plus cDNA Synthesis Mix	5 μl
Random 6 mers Primer (100μM) *1,*2	2 μl
Template RNA	Total RNA ≤5μg / Poly(A)+RNA ≤1μg
RNase free water	Up to 20 μl *3

*1: When the synthesized cDNA is intended for Real-Time PCR–based gene expression analysis, it is recommended to add 2 μl Random 6 mers Primer for reverse transcription. If the target gene abundance is low, the template is complex (e.g., high GC content), or the results are suboptimal, the amount of Random 6 mers Primer may be increased to 4 μl.

*2: A Gene-Specific Primer (GSP) may also be used; in this case, the final concentration in the reaction system should be 0.1 μM.

2) Run the cDNA Synthesis Reaction Setup as below.

Temperature	Time
30°C	5 min*1
50°C*2	15 min*3
85°C	5 min*4
4°C	-

*1: When using Random 6 mers Primer for reverse transcription, a pre-incubation at 30 °C for 5 min is recommended before starting the reaction to allow sufficient annealing between random primers and the template, thereby improving reverse transcription efficiency. The annealing time may be adjusted within 0-10 min according to experimental needs. If 30 °C for 5 min does not yield optimal results, the annealing time may be extended to 10 min.

*2: Evo Super M-MLV Plus exhibits robust extension performance even with templates containing complex secondary structures. The reverse transcription temperature is typically set to 50 °C to obtain optimal results and may be adjusted within 42 °C-55 °C as needed. When using Gene-Specific Primers, the reverse transcription temperature can be adjusted within 50 °C-60 °C.

*3: In most cases, a 15 min reverse transcription reaction yields satisfactory results. The reaction time may be adjusted within 5-15 min depending on experimental requirements.

*4: Under normal conditions, incubation at 85 °C for 5 min is sufficient to completely inactivate the reverse transcriptase. The inactivation time may be adjusted within 2-5 min as needed.

3) The cDNA solution obtained above can be used directly for subsequent Real Time PCR amplification. The volume of cDNA added should not exceed 1/10 of the total qPCR reaction volume.

Accurate Biotechnology (Hunan) Co., Ltd
No.1 JinYang Road
WangCheng District, Changsha City, Hunan Province, China
service@agbio.com.cn
+86 400 767 6022
en.agbio.com.cn
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