艾科瑞生物
Accurate Biology

Instruction for Use

Evo M-MLV RT Kit for qPCR

AG11707
Version.V4E1

Research Use Only
Not For Diagnosis Procedures

1. Description

This product is a reverse transcription reagent specifically designed for Real-Time RT-PCR. It incorporates the high-processivity Evo M-MLV reverse transcriptase, enabling rapid and efficient cDNA synthesis in a short time. The resulting cDNA is fully compatible with both dye-based and probe-based qPCR assays, and can be used with appropriate downstream reagents to achieve high-performance gene expression analysis tailored to different experimental objectives.

2. Kit Information

Kit Name	Cat. No	Specification
Evo M-MLV RT Kit for qPCR	AG 11707	200 rxns / 10 μl

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
Evo M-MLV RTase Enzyme Mix*1	100 μl
5X RTase Reaction Buffer Mix II*2	400 μl
Oligo dT (18T) Primer (50 μM)	100 μl
Random 6 mers Primer (100 μM)	400 μl
RNase Free Water	1 ml x 2 Pcs

*1: Contains Evo M-MLV RTase, RNase Inhibitor.

*2: Contains dNTP.

5. Precautions

1) Before use, briefly centrifuge the Evo M-MLV RTase Enzyme Mix to collect all enzyme solution at the bottom of the tube and prevent loss of enzyme during handling.
2) When setting up multiple reactions, first prepare a master mix of all required reagents, then aliquot it into individual reaction tubes.
3) All reaction mixtures should be prepared on ice.

6. Protocol

Prepare the RT mix as below

Components	Input Volume[*2]
5X RTase Reaction Buffer Mix II	2 μl
Evo M-MLV RTase Enzyme Mix	0.5 μl
Oligo dT (18T) Primer (50 μM)[*1]	0.5 μl
Random 6 mers Primer (100 μM)[*1]	0.5 μl
Total RNA[*3]	-
RNase free water	Up to 10 μl

Reaction Program:

Temperature	Time
37°C[*4]	15 min
85°C	5 sec
4°C	Hold[*5]

1) When using the SYBR Green qPCR method, add 0.5 μl Random 6 mers Primer (100 μM).
   When using the probe-based qPCR method, add 2 μl Random 6 mers Primer (100 μM).
   Alternatively, the primer mix can be omitted and individual primers may be used as follows:
   Oligo dT (18T) Primer: 25 pmol per 10 μl reaction
   Random 6 mers Primer: 50 pmol per 10 μl reaction (for SYBR Green qPCR)
   Random 6 mers Primer: 200 pmol per 10 μl reaction (for probe-based qPCR)
   Gene-Specific Primer: 2.5 pmol per 10 μl reaction (adjustable within 1-5 pmol)
2) To ensure accurate preparation of the reverse transcription reaction, it is recommended to prepare a master mix of all components first, aliquot into reaction tubes, and finally add the RNA samples. The reaction volume may be adjusted as required.
3) The amount of RNA can be adjusted as needed. In a 10 μl reverse transcription reaction, use up to 500 ng total RNA for SYBR Green qPCR and up to 1 μg total RNA for probe-based qPCR.
4) When using a Gene-Specific Primer, the reaction may be performed at 42 °C for 15 min. To reduce non-specific amplification, the reaction temperature can be increased to 50°C.
5) 5: If the reaction product is used immediately for subsequent qPCR, it may be kept at 4°C or on ice. For short-term storage, store at -20°C; for long-term storage, store at -80°C.

7. Quantitative PCR Reaction Analysis

The reaction mixture obtained above can be directly used for subsequent quantitative PCR. Ensure that the added volume does not exceed 1/10 (V/V) of the qPCR reaction volume.

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