艾科瑞生物
Accurate Biology

Instruction for Use

Evo Super M-MLV One Step RT-PCR Mix (dye plus)

AG11625
Version.V1E1

Research Use Only
Not For Diagnosis Procedures

1. Description

This product is a one-step RT-PCR kit that uses RNA as the template, in which reverse transcription and PCR amplification are performed sequentially in a single reaction system. The workflow is simple and rapid, requiring no additional reagent addition during the reaction, thereby effectively reducing the risk of contamination.

The kit has been optimized for single-tube operation, integrating the reverse transcriptase, DNA polymerase, and reaction buffer into one system for ease of use. It combines the high-performance Evo Super M-MLV reverse transcriptase, Pro Taq HS DNA polymerase, and auxiliary proteins, with an optimized buffer formulation. As a result, the kit offers broad compatibility with different RNA templates, high amplification efficiency and yield, and high detection sensitivity, making it well suited for RNA amplification and analysis. In addition, electrophoresis loading dye is included in the reaction mix, allowing PCR products to be directly loaded onto a gel after amplification, enabling faster result visualization.

2. Kit Information

Kit Name	Cat. No	Specification
Evo Super M-MLV One Step RT-PCR Mix (dye plus)	AG 11625	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
2X One Step RT-PCR Mix (dye plus)	1.25 ml
RNase Free Water	1 ml x 2 Pcs

5. Precautions and Preparation

1) During operation, take precautions to prevent RNase contamination from the working environment and handling procedures.
2) The reaction mixture should be prepared on ice, and the assembled reaction should be immediately placed into the PCR instrument for incubation.
3) Reagents & Consumables: Primers, RNase-free water, RNase-free 1.5 mL microcentrifuge tubes, RNase-free PCR tubes, and RNase-free pipette tips.
4) Instruments: PCR thermal cycler, pipettes, vortex mixer, mini benchtop centrifuge, electrophoresis system, and gel imaging system.

6. Protocol

1) Prepare the reaction mix as below on ice.

Components	Final Concentration	Input Volume of 50 μl System
2X One Step RT-PCR Mix (dye plus)	1X	25 μl
Primer F（10μM）	0.4 μM	2 μl
Primer R（10μM）	0.4 μM	2 μl
Total RNA	≤1μg	-
RNase free water	-	Up to 50 μl *3

*1: Primers are typically used at a final concentration of 0.4 μM and may be adjusted within the range of 0.2–1.0 μM according to experimental requirements.

*2: In a 50 μl reverse transcription reaction system, the recommended amount of total RNA should not exceed 1 μg.

2) Run the PCR Reaction Setup Program as below.*1*2

Step	Temperature	Time	Number of Cycles
Reverse Transcription	55°C*3	30 min*4	1
Pre-Denaturation	94°C	2 min	1
Denaturation	94°C	30 sec	30 ~ 40*5
Annealing	56°C	30 sec	
Extension	72°C	1 min/kb	
Final Extension	72°C	5 min	1

*1: It is recommended to first use the three-step PCR cycling program listed above. If satisfactory results are not obtained, further optimization of the reaction conditions may be performed.

*2: When the primer Tm is high or the three-step PCR yields suboptimal results, a two-step PCR protocol may be attempted (refer to the Appendix for the two-step PCR program).

*3: A reverse transcription temperature of 55 °C generally improves reaction specificity and may be adjusted within the range of 50–60 °C as needed.

*4: A reverse transcription time of 30 min typically yields optimal results and may be adjusted within the range of 15–30 min depending on experimental requirements.

*5: If the amplification bands are weak or the template amount is low, the number of PCR cycles may be increased appropriately, but it is not recommended to exceed 40 cycles.

7. Result Analysis

After the reaction is complete, load 2–5 μl of the PCR product onto an agarose gel to analyze the product yield and specificity by electrophoresis.

Appendix. 2 Step RT-PCR Reaction Program

Step	Temperature	Time	Number of Cycles
Reverse Transcription	55°C	30 min	1
Pre-Denaturation	94°C	2 min	1
Denaturation	98°C	10 sec	30 ~ 40
Extension	68°C	1 min/kb	
Final Extension	72°C	10 min	1

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