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Accurate Biology

Instruction for Use

Evo M-MLV RT Mix Kit with gDNA Clean for qPCR Ver.2

AG11728
Version.V3E1

Research Use Only
Not For Diagnosis Procedures

1. Description

This product is a Reverse Transcription reagent kit that utilizes M-MLV Reverse Transcriptase, and the resulting cDNA can be directly used for qPCR detection. The 5X Evo M-MLV RT Reaction Mix Ver.2 contains all the components required for Reverse Transcription reactions. It has been optimized for the quantities of Oligo dT (18T) Primer and Random 6 mers Primer, making the Reverse Transcription product compatible with both hybridization and probe qPCR analysis, enabling efficient gene expression analysis.

During downstream quantitative PCR experiments, the presence of genomic DNA (gDNA) mixed in with Total RNA can potentially lead to false-positive results when used as a template for PCR amplification. The gDNA Clean Reaction Mix Ver.2 included in this product effectively removes residual genomic DNA, ensuring the accuracy of quantitative results. Additionally, this product includes NRT Control Reaction Mix, which can be used to prepare negative control reactions without Reverse Transcriptase.

This product is suitable for different operating methods, allowing customers to choose according to their needs:

1. Two-step method (remove gDNA first, then perform Reverse Transcription):

This method is recommended if there is excessive residual gDNA in the extracted RNA or if a higher gDNA removal efficiency is required.

2. All-in-one (gDNA removal and Reverse Transcription reactions performed simultaneously in one tube):

This method can be selected when RNA quality is high (minimal gDNA residue), or when RNA concentration is high and target gene copy numbers are high. It is convenient and quick, simplifying experimental procedures and shortening the experimental duration.

2. Kit Information

Kit Name	Cat. No	Specification
Evo M-MLV RT Mix Kit with gDNA Clean for qPCR Ver.2	AG 11728	100 rxns / 20 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
gDNA Clean Reaction Mix Ver.2	240 µl
5X *Evo M-MLV* RT Reaction Mix Ver.2*1	400 µl
NRT Control Reaction Mix*2	80 µl
RNase Free Water	1 ml x 2 Pcs

*1: This solution contains Evo M-MLV RTase, RNase Inhibitor, dNTPs, Oligo dT (18T) Primer, and Random 6 mers Primer.

*2: This solution does not contain Evo M-MLV RTase. However, all other components are identical to those in the 5X Evo M-MLV RT Reaction Mix Ver.2.

*3: Before using any of the solutions, briefly centrifuge them and then gently mix them by pipetting up and down. During this process, try to avoid bubble formation as much as possible. Then proceed with usage.

5. Protocol

5.1 RNA Reverse Transcription

This product offers two usage methods, and customers can choose according to their needs. The operations are as follows:

Method 1 - Two-step (remove gDNA first, then perform Reverse Transcription):

1) gDNA Removal

Prepare thegDNA Removal mix as below on ice, and run the gDNA removing program as following.

Components	Reaction System 1	Reaction System 2
gDNA Clean Reaction Mix Ver.2	2 μl	2 μl
Total RNA *2	-	-
RNase free water	Up to 10 μl *3	Up to 16 μl *3

*1: It is recommended to first prepare the reagents, excluding RNA, into a premix solution, mix thoroughly, and then add the RNA template.

*2: The amount of RNA can be adjusted according to the requirements. It is recommended to dissolve the extracted RNA in RNase-free water. When performing qPCR amplification in a 20μl reverse transcription system, for SYBR-based assays, a maximum of 1μg Total RNA can be used, while for probe-based assays, a maximum of 2μg Total RNA can be used.

*3: Typically, a 10μl system is chosen for gDNA removal. If the RNA concentration is low, you can also choose System 2 and increase the amount of RNA added. Adjust the volume to 16μl with RNase-free water for the reaction. At the same time, omit the addition of RNase-free water in step 2) during the reverse transcription reaction.

Reaction Program:

Temperature	Time
42°C	2 min
4°C	-

2) Reverse Transcription

Prepare the RT mix as below

Components	Reaction System 1	Reaction System 2
Reaction Mix from Step 1) gDNA removal	10 μl	16 μl
5X *Evo M-MLV* RT Reaction Mix Ver.2 *4 *5	4 μl	4 μl
RNase free water	6 μl	-
Total	20 μl	20 μl

*4: If using System 1 for the reaction setup, prepare the reverse transcription reaction by pre-mixing 5X Evo M-MLV RT Reaction Mix Ver.2 and RNase-free water to form a Master Mix. Then, aliquot 10μl of this Master Mix into the 10μl reaction mixture prepared in step 1), gently mix, and proceed with the reverse transcription reaction. If using System 2 for the reaction, directly add 4μl of 5X Evo M-MLV RT Reaction Mix Ver.2 to the 16μl reaction mixture prepared in step 1).

*5: NRT Control refers to the negative control reaction without reverse transcriptase. It can be prepared according to actual needs. For preparation, simply replace the solution with NRT Control Reaction Mix.

Note: The cDNA product is recommended to be stored at -20°C or -80°C for preservation.

Reaction Program:

Temperature	Time
37°C	15 min
85°C	5 sec
4°C	-

Method 2 - All-in-one (gDNA removal and Reverse Transcription reactions performed simultaneously in one tube):

When RNA quality is high (minimal gDNA residue), or when RNA concentration is high and target gene copy numbers are high, the removal of gDNA and reverse transcription can be combined into a single step operation.

Prepare the RT mix as below

Components	Reaction System 1
gDNA Clean Reaction Mix Ver.2	2 µl
5X *Evo M-MLV* RT Reaction Mix Ver.2 *7 *8	4 µl
Total RNA *9	-
RNase free water	Up to 20 µl

*7: It is recommended to first prepare the components, excluding RNA, into a premix solution, mix thoroughly, and then add the RNA template.

*8: NRT Control refers to the negative control reaction without reverse transcriptase. It can be prepared according to actual needs. To prepare, simply replace the solution with NRT Control Reaction Mix.

*9: The amount of RNA can be adjusted according to the requirements. It is recommended to dissolve the extracted RNA in RNase-free water. When performing qPCR amplification in a 20 µl reverse transcription system, for SYBR-based assays, a maximum of 1 µg Total RNA can be used, while for probe-based assays, a maximum of 2 µg Total RNA can be used.

Reaction Program:

Temperature	Time
37°C	15 min
85°C	5 sec
4°C	-

Note: The cDNA product is recommended to be stored at -20°C or -80°C for preservation.

5.2 Quantitative PCR Reaction Analysis

The reaction mixture obtained from step 2) can be directly used for subsequent quantitative PCR reactions, with its volume not exceeding 1/10 (V/V) of the total volume of the quantitative PCR reaction. The cDNA product obtained from this reagent kit's reverse transcription is not suitable for amplifying long gene fragments. If long fragment amplification is needed, other products from our company can be considered.

Note: If low fluorescence signals occur when using the cDNA directly for quantitative PCR reactions, try diluting the cDNA solution 2 to 10 times before conducting the experiment

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