M-MLV Reverse Transcriptase

Store at -20°C

Description

The Omega Bio-tek's M-MLV Revers Transcriptase is Moloney Murine Leukemia Virus Revers Transcriptase, it is an RNA-dependent DNA polymerase that can be in cDNA synthesis with long messenger RNA templates. The M-MLV RTase that has been engineered to reduce RNase H activity and provide increased themal stability. The enzyme is used to synthesize cDNA at a temperature range of 37-42°C, providing increased specificity, higher yields of cDNA, and more full-length product than other reverse transcriptase.

Kit components

Cat.No.	TQ2401-01	TQ2401-02
M-MLV Reverse Transcriptase	5000U	50000U
5× RT Buffer	150μl	2×1ml

Quality Control

To test for RNase, DNase and Endonuclease activity found there is no contaminant .The product is tested functionally 10ng and 1μg of chicken liver total RNA as the template, transcription product 2% for amplification 1077bp β-actin target(40 cycles).

Important Guidelines

- Hight quality intact RNA is essential for successful full-length cDNA synthesis. We recommend the E.Z.N.A.® total RNA Kit or RNA-Solv® Reagent for isolation of total RNA.
- RNA should be devoid of any RNase contamination and aseptic conditions should be maintained. RNase Inhibitor has been added to the system to safeguard against degradation of target RNA due to ribonuclease contamination of the RNA preparation.
- You can preheated the thermal cycler to 42°C or 37°C before setting up the reaction.
- Keep all components, reaction buffer, dNTPs and samples on ice. After preparation of the reaction, transfer them to the preheated thermal cycler and immediately start the RT-PCR program.
- Efficient cDNA synthesis can be accomplished in a 60 min incubation at 42°C with oligo(dT) or GSPs, and 37℃ with random 6 mers primer.

Reagents to Be Supplied by User

- *RNase inhibitor*

  RNase inhibitor is a 49.6kDa protein that strongly inhibits RNase A, B, C, as well as human placental Rnases. For best results, we strongly recommend using RNase inhibitor to minimize the risk of RNA degrdation during exprimental setup. RNase inhibitor(catalog no. TQR01-01/02) is commonly supplied at a concentration of 40 units/μl.
- *Primers*

  Gene-specific primers is commonly used at a final concentration of 0.1-1.0 μM, Oligo(dT) and random 6 mers primers are commonly used at a final concentrtion of 10 μM in the revers-transcription reaction.

For RT-PCR

- *Taq polymerase(catalog no. TQ2100-00/01/02) or hotstart Taq polymerase(catalog no. TQ2102-00/01), PCR buffer, primers, reagents, and additional nucleotides for PCR*

Protocol

This is the standard protocol for first-strand cDNA synthesis using 10 ng to 2 μg RNA and Omega Bio-tek M-MLV revers transcriptase. Template cDNA generated using Omega Bio-tek M-MLV revers transcriptase is suitable for use in standard PCR and real-time PCR.

1. Thaw template RNA on ice. Thaw the primers solution(supplied by users), 5×RT Buffer, dNTPs, and Nuclease-Free Water at room temperature(15-25℃). Store on ice immediately after thawing. Mix each solution by vortexing, and centrifuge briefly to collect residual liquid from the sides of the tubes.

2. **Add the following to a 0.2 ml thin-walled PCR tube (nuclease-free).** For multiple reactions, you can prepare a master mix to minimize reagent loss and enable accurate pipetting.

Component	Volume
10 ng-2 μg total RNA	n μl
Primer	
Gene-specific primer(GSPs 10 μM),	1 μl
or oligo(dT)$_{15}$(50 μM), or random 6 mers(50 μM)	
10 mM dNTP mix	1 μl
Nuclease-Free Water	to 18 μl

3. **Incubate at 65-70°C for 5 min, then place on ice for at least 2min.**

4. prepare the following cDNA synthesis mix, adding each component in the indicated order.

Component	Volume
5× RT Buffer	5 μl
M-MLV Reverse Transcriptase (RNase H-, 200U/μl)	1 μl
RNase Inhibitor(40U/μl)	1 μl

5. Add 7 μl of cDNA synthesis mix to each RNA/primer mixture, mix gently, and collect by brief centrifugation. Incubate as follows.

- Oligo(dT)$_{15}$ or gene-specific primed: 60 min at 42°C
- Random 6 mers primed: 60 min at 37°C

6. **Terminate the reactions at 85℃ for 5 min. Chill on ice.**

7. cDNA synthesis reaction can be stored at -20°C or used for PCR immediately.

Amplification of Target cDNA

The first-strand cDNA obtained in the synthesis reaction may be amplified directly using PCR. We recommend using 1-3 μl the first-strand cDNA reaction for PCR. However, for some targets, increasing the amount of first-strand cDNA reaction up to 10 μl in PCR may result in increased product yield. For PCR amplication we recommend using Omega Bio-Tek Taq DNA polymerase or Omega Bio-Tek perfectstart Taq DNA polymerase.