M-MLV Frist Strand cDNA Synthesis Kit

Store at -20°C

Description

The Omega Bio-Tek's M-MLV Frist Strand cDNA Synthesis Kit is optimized to synthesize first-strand cDNA from pruified poly(A)+ or total RNA. This kit is a version of M-MLV RT 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. RNA targets from 100bp to > 12kb can be detected with this kit. The amount of starting material can range from 10ng to 2μg of total RNA.

cDNA synthesis is perfromed in the first step using either total RNA or poly(A)+-selected primed with oligo(dT), random primers, or a gene-specific primer. In the second step, PCR is performed in a separate tube using primers specific for the gene of interest. For the PCR reaction, we recommend use Omega Bio-Tek Taq DNA polymerase or perfectstart Taq DNA polymerase for increased specificity.

Kit components

Cat.No.	TQ2501-01	TQ2501-02
Preps	20 preps	100 preps
5 x RT Buffer	150 μl	750 μl
Oligo(dT)$_{15}$ (50μM)	20 μl	100 μl
Random 6 mers (50μM)	20 μl	100 μl
dNTPs (10mM)	20 μl	100 μl
M-MLV Reverase Transcriptase (RNase H-, 200U/μl)	20 μl	100 μl
RNase Inhibitor (40U/μl)	20 μl	100 μl
Nuclease-Free Water	500 μl	2 ml

Quality Control

To test for RNase, DNase and Endonuclease activity found there is no contaminant .The product is tested functionally 100ng and 1μg of chicken liver total RNA as the template, transcription product 1 μl for amplification 1077bp ß-actin target(40 cycles).

Important Guidelines

RNA

- Hight quality intact RNA is essential for successful full-length cDNA synthesis.
- For low copy-number genes or longer target , use more starting material(>20ng 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.
- We recommend the E.Z.N.A.® total RNA Kit or RNA-Solv® Reagent for isolation of total RNA.
- Small amounts of genomic DNA in the RNA preparation may be amplified along with the target cDNA. If you application requires removal of all genomic DNA from your RNA sample, we recommend using DNase I(catalog no. E1091-01/02) to digest genomic DNA.

Primers

The first-strand cDNA synthesis reaction can be primed using random 6 mers, oligo(dT), or gen-specific primers(GSPs).

- Random 6 mers are the most nonspecific priming method, and are typically used when the mRNA is difficult to copy in its entirety. With this method, all RNAs in a population are templates for first-strand cDNA synthesis, and PCR primers confer specificity during PCR. To maximize the size of cDNA, you should determine the ratio of random hexamers to RNA empirically for each RNA preparation.
- Oligo(dT) is a more specific priming method, is used to hybridize to 3'poly(A) tail, which are found in the vast majority of eukaryotic mRNA. Since poly(A) RNA constitutes approximately 1%-2% of total RNA, the amount and complexity of cDNA is considerably less than with random 6 mers.

- The most specific priming method uses a gene-specific primer for the sequence of interest. First-strand synthesis can be primed with the PCR primer that hybridizes nearest to the 3' terminus of the mRNA.

Note: For most RT-PCR applications, 1μl(50μM) of random 6 mers per reaction is adequate. Increasing 6 mers may increase yield of small PCR products(<500bp), but may decrease the yield of longer PCR products and full-length transcripts. Oligo(dT) is recommended over random 6 mers or GSPs when performing RT-PCR with new mRNA targets. Oligo(dT) produces an RT-PCR product more consistently than random 6 mers or GSPs. Some GSPs fail to prime cDNA synthesis even though they work in PCR on DNA templates. If GSPs priming fail in RT-PCR, repeat first-strand synthesis using oligo(dT) as the primer.

Reactions

- You can preheated the thermal cycler to 42°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.

Protocol

1. **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	1 μl
Gene-specific primer(GSPs 10 μM),	
or oligo(dT)$_{15}$(50 μM), or random 6 mers(50 μM)	
10 mM dNTP mix	1 μl
Nuclease-Free Water	to 18 μl

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

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

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

4. 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

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

6. 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 Taq DNA polymerase or omega perfectstart Taq DNA polymerase.