

TonkBio™ First-Strand cDNA Synthesis Kit

Product	Size	Cat. No.
TonkBio™ First-Strand cDNA Synthesis Kit	50 reactions	TB30001A
Includes:		
TonkBio™ M-MLV (200 U/ μ L)		120 μ L
OligodT primer		120 μ L
Random primer		120 μ L
5 \times Reaction buffer		500 μ L
dNTP mix (10 mM each)		240 μ L
RNase Inhibitor		60 μ L
RNase-free H ₂ O		2 \times 1 mL

Storage and Stability

Store all system components at -20°C for one year.

Description

The TonkBio™ First Strand cDNA Synthesis Kit for RT-PCR is optimized to efficiently synthesize first strand cDNA from mRNA or total RNA templates. The kit uses M-MLV reverse transcriptase with lower RNase H activity, which avoids degradation of RNA when RNA-DNA hybrid forms in the progress of cDNA synthesis. It is suitable for synthesis of cDNA up to 9 kb. The cDNA products could be used directly in a variety of downstream applications, including PCR, qPCR, gene clone.

Caution

1. For research use only.
2. Use RNase-free reagents during RNA extraction and cDNA synthesis process.
3. NO repeated freezing and thawing RNA. RNA should be dissolved in ice-water mixture.
4. The reverse transcription reaction products should be stored at -20°C.
5. Wear gloves when handling RNA and all reagents as skin is a common source of RNases..

Standard cDNA Synthesis

1. First-strand cDNA synthesis

Store all components of the kit on ice after thawing, mixing and brief centrifugation.

- 1) Add the following components into a sterile, nuclease-free tube on ice as shown:

Total RNA / mRNA	0.01 -5 μ g/ 10 pg-0.5 μ g
OligodT primer / Random primer	1 μ L / 20 pmol
RNase free H ₂ O	up to 12.5 μ L

- 2) Incubate at 65°C for 5 minutes. Chill on ice, spin down and place the vial back on ice.

3) Add the following components:

5 × Reaction buffer	4 µL
RNase Inhibitor	0.5 µL
dNTP mix (10 mM each)	2 µL
TonkBio™ M-MLV (200 U/ µL)	1 µL

Mix gently and centrifuge briefly.

4) For a reaction using OligodT or gene specific primers, incubate at 42°C for 1 hour. For random hexamer primed synthesis incubate at 25°C for 5 minutes followed by 42°C for 1 hour.

5) Terminate the reaction by heating at 70°C for 5 minutes.

***All operations must be done on ice.**

Time of reverse transcription can be adjusted at 30-60 minutes. And extending the reverse transcription time is helpful in obtaining longer cDNA.

2. PCR Amplification of First Strand cDNA

The product of the first strand cDNA can be used directly in PCR or qPCR. The volume of the first strand cDNA synthesis reaction mixture should be no more than 1/10 of the total PCR reaction volume. For qPCR amplification, the amount of the total RNA is no more than 1µg.

PCR system recommended:

The first-strand cDNA	1-2 µL
Primer-F (10 µM)	1 µL
Primer-R (10 µM)	1 µL
2× PCR Master Mix	25 µL
ddH ₂ O	up to 50 µL

Troubleshooting Guide

1. NO or low product of RT PCR

- 1) Degradation of RNA template. RNA Purity and integrity is important to obtain full length cDNA. Users have to make gel electrophoresis detection to ensure no degradation occurred. And avoid repeated freezing and thawing RNA.
- 2) Low concentration of RNA template. Trace amount of agents used in RNA purification may remain in solution and inhibit cDNA synthesis. Suggest to wash RNA with 75% ethanol (prepared by DEPC water) carefully.
- 3) Incorrect primers. Select random primers or specific primers instead of OligodT primer with bacterial RNA or RNA without a poly A tail.
- 4) GC rich template. If the RNA template is GC-rich or contains secondary structures, choose random primers and increase the reaction temperature of transcription up to 55°C.

2. RT-PCR product longer than expected

RNA sample is contaminated with DNA. To avoid genomic DNA contamination, User could use DNase I to digest RNA sample before transcription or design primers on exon-intron boundaries.

3. Product of RT-PCR in negative control

RNA template is contaminated with DNA. Perform DNaseI digestion prior reverse transcription.