MMLV First-Strand Synthesis Kit

Cat No. MB301-0050
Size: 50 reactions
Store at -20°C

Description

In molecular biology and biochemistry, the reverse transcriptase, also known as RNA-dependent DNA polymerase, is a DNA polymerase enzyme that transcribes single-stranded RNA into double-stranded DNA. It also helps in the formation of a double helix DNA once the RNA has been reverse transcribed into a single strand cDNA.
The MMLV First-Strand Synthesis Kit is designed for reverse transcription using TaqMan probe for detection. This product uses MMLV Reverse Transcriptase, which has an excellent extension ability and can efficiently synthesize cDNA in a short time period. The system can be used with as little as 1-5ng total RNA or 1-500ng mRNA for synthesis.

Components

Component Amount	Amount (50-rxn size)
Oligo(dT)20 (50mM)	50µl
Random hexamers (50 ng/µl)	250µl
5X 1st strand buffer	1 ml
DTT (0.1M)	500µl
dNTP mix (10mM)	250µl
MMLV Reverse Transcriptase(200U/µl)	50µl

* This product is distributed for laboratory research only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

Quality Control

The product has passed the genomic DNA quantitative PCR function test.
Kinetic analysis confirmed that the reagent concentration of the target gene with a linear dose-dependent decline could be detected with human genomic DNA at as low as 10pg . The DNase, RNase, and exonuclease are absent.

Protocol

cDNA Synthesis
1. Set up reaction tubes/plates on ice.
2. Add the following components in any order to each reaction vessel.

Component	Volume
Oligo (dT)20 (50mM),	1µl
Total RNA or 1ng-500ng mRNA,	1ng-5µg
dNTP mix (10mM each)	1µl

Add DEPC-treated water to 12µl.

3. Incubate the mixture at 65°C for 5 minutes, then place on ice for at least 1 minute.
4. Collect the reaction by brief centrifugation. Add the following contents:

Component	Volume
5X 1st strand synthesis buffer	4µl
0.1M DTT	2µl
Recombinant RNase Inhibitor (40U/µl)	1µl

(Note: If the RNA is less than 50ng, add Recombinant RNase Inhibitor to remove RNase)
5. Mix the components briefly*, and incubate at 37°C for 2min.
6. Add 1µl (200U) MMLV Reverse Transcriptase at RT and mix.
(In case of the random hexamers, incubate at 25°C for 10min.)
7. Incubate at 37°C for 50min.
8. Heat at 70°C for 15min to stop the reaction.
The cDNA synthesized in the first-strand reaction may be amplified directly using PCR. If the amplified PCR product is longer than 1kb, remove the RNA template from the cDNA:RNA hybrid molecule by digestion with 1µl(2unit) E.coli RNase H at 37°C for 20min to remove the RNA template.

* If template RNA is less than 1ng, decrease the reaction volumes of MMLV transcriptase to 0.25µl (50unit), then add DEPC-treated water to 20µl.

Quantitative Real Time Polymerase Chain Reaction
(Q-PCR/ qPCR/ qrt- PCR)
For the quantitative RT-PCR, the total RNA between 10pg and 1µg used was reversely transcribed in 5µl undiluted or 10µl diluted cDNA. It should be noted that when testing undiluted cDNA in high abundance genes, it may lead to a very low CT value as a result, thus affecting the quantitative accuracy. To dilute the cDNA, the template will attain more accurate results.

Plasmid and Genomic DNA
Use 100 pg to 1µg of genomic DNA or 10–107 copies of plasmid DNA in a 10µl volume. Note that 1µg of plasmid DNA contains 9.1 X1011 copies divided by the plasmid size in kilobases.

Guidelines and Recommendations
This product is designed to perform the reverse transcription of RNA to cDNA and subsequent amplification. It is important to use the highly pure RNA sample for better cDNA synthesis yields. So, it is essential to inhibit cellular RNase activity and prevent contamination with RNase derived from equipment and solutions used.
Extra precaution should be taken during the sample preparation, including use of clean disposable gloves, dedication of a table exclusively for RNA preparation, and avoidance of unnecessary speaking during assembly, to prevent the RNase contamination from operator's sweat or saliva.

Trouble-Shooting

Problem	**Possible Cause**	**Solution**
Signals are present in no-template controls, and/or multiple peaks are present in the melting curve graph	Template or reagents are contaminated by nucleic acids (DNA, cDNA)	Use melting curve analysis if possible, and/or run the PCR products on a 4% agarose gel after the reaction to identify contaminants. To reduce the risk of contamination, take standard precautions when preparing your PCR reactions.
	Primer dimers or other primer artifacts are presen	Use melting curve analysis to identify primer dimers at their lower melting temperature if possible. Use validated primer sets or design primers/probes using dedicated software programs or primer databases. Check the purity of your primers by gel electrophoresis. If agarose gels are used, we recommend cooling the gels before visualization with intercalating dyes.
No amplification curve appears on the qPCR graph	There is no PCR product	Run the reaction on a gel to determine whether PCR worked. Then proceed to the troubleshooting steps below.
No PCR product is evident, either in the qPCR graph or on a gel	The protocol was not followed correctly	Verify that all steps have been followed and the correct reagents, dilutions, volumes, and cycling parameters have been used.