Maxima H Minus First Strand cDNA Synthesis Kit

Catalog Number K1651, K1652

Pub. No. MAN0012720 **Rev.** B00

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support.**

Contents

Cat. No.	Contents	Amount	Storage
K1651 (20 reactions)	Maxima H Minus Enzyme Mix	25 µL	-25 °C to -15 °C
	5X RT Buffer 250 mM Tris-HCl (pH 8.3 at 25 °C), 375 mM KCl, 15 mM $MgCl_2$, 50 mM DTT	150 µL	
	10 mM dNTP Mix	50 µL	
	Oligo$(dT)_{18}$ Primer, 100 µM	25 µL	
	Random Hexamer Primer, 100 µM	25 µL	
	Water, nuclease-free	1.25 mL	
K1652 (200 reactions)	Maxima H Minus Enzyme Mix	120 µL	
	5X RT Buffer 250 mM Tris-HCl (pH 8.3 at 25 °C), 375 mM KCl, 15 mM $MgCl_2$, 50 mM DTT	500 µL	
	10 mM dNTP Mix	250 µL	
	Oligo$(dT)_{18}$ Primer, 100 µM	120 µL	
	Random Hexamer Primer, 100 µM	120 µL	
	Water, nuclease-free	2 x 1.25 mL	

Description

The Thermo Scientific™ Maxima™ H Minus First Strand cDNA Synthesis Kit is a complete system for efficient synthesis of first strand cDNA. The kit uses the Maxima H Minus Reverse Transcriptase (RT), an advanced enzyme derived by *in vitro* evolution of M-MuLV RT. The enzyme features high thermostability and lacks the RNase H activity. The Maxima H Minus First Strand cDNA Synthesis Kit is capable of synthesizing cDNA up to 20 kb from a wide range of total RNA amounts (1 pg to 5 µg) at elevated temperatures (45-65 °C) superceeding other systems in ability to prepare full length cDNA. Due to increased synthesis rate reaction can be completed in 30 min. Maxima H Minus Enzyme Mix contains Maxima H Minus Reverse Transcriptase and Thermo Scientific™ RiboLock™ RNase Inhibitor. The recombinant RiboLock RNase Inhibitor effectively protects RNA from degradation by RNases A, B and C at temperatures up to 55 °C.

The kit is supplied with both oligo$(dT)_{18}$ and random hexamer primers. Random hexamer primers bind non-specifically and are used to synthesize cDNA from all RNAs in total RNA population. The oligo$(dT)_{18}$ primer selectively anneals to the 3'-end of poly(A) RNA, synthesizing cDNA only from poly(A) tailed mRNA. Gene-specific primers may also be used with the kit to prime synthesis from a specified sequence.

The synthesized cDNA can be used directly in PCR, qPCR or in second strand cDNA synthesis.

Important Notes

Avoiding ribonuclease contamination

RNA purity and integrity are essential for synthesis of full-length cDNA. RNA can be degraded by RNase A, which is a highly stable contaminant found in any laboratory environment. To prevent contamination both the laboratory environment and all prepared solutions must be free of RNases.
General recommendations to avoid RNase contamination:

- Use certified nuclease-free labware or DEPC-treat all tubes and pipette tips to be used in cDNA synthesis.
- Wear gloves when handling RNA and all reagents, as skin is a common source of RNases. Change gloves frequently.
- Use RNase-free reagents, including high quality water (e.g., Water, nuclease-free, #R0581).
- Keep the kit components tightly sealed when not in use. Keep all tubes tightly closed during the reverse transcription reaction.

Template RNA

Total cellular RNA isolated by standard methods is suitable for use with the kit. Purified RNA must be free of salts, metal ions, ethanol and phenol to avoid inhibiting the cDNA synthesis reaction.
For RT-PCR applications, template RNA must be free of DNA contamination. Prior to cDNA synthesis, RNA can be treated with DNase I, RNase-free (#EN0521) or dsDNase (#EN0771) to remove trace amounts of DNA.
Always perform a RT-minus negative control reaction, which includes all components for RT-PCR except the Maxima H Minus Enzyme Mix.

RNA sample quality

Assess RNA integrity prior to cDNA synthesis. Total eukaryotic RNA can be analyzed by agarose gel electrophoresis followed by ethidium bromide staining. The RNA is considered to be intact, if both 18S and 28S rRNA appear as sharp bands after electrophoresis of total RNA. The 28S rRNA band should be approximately twice as intense as the 18S rRNA. Any smearing of rRNA bands is an indication of degraded mRNA. If this occurs, a new sample of total RNA should be prepared. Alternatively, total RNA can be analyzed using a bioanalyzer (e.g., Agilent 2100) which provides quantitative information about the general state of the RNA sample, the RNA integrity number (2). A reference gene/target gene 3':5' integrity assay (3) can also be used to determine the integrity of the RNA sample.

RNA quantity

- Use 1 pg - 5 µg of total RNA, 0.1 pg - 500 ng of poly(A) mRNA or 0.01 pg – 500 ng of specific RNA transcript to generate first strand cDNA as the initial step of a two-step RT-PCR protocol.
- Use 1 µg of isolated mRNA to generate first strand cDNA for second-strand synthesis and subsequent cloning reactions.

Primers

Synthesis of first strand cDNA can be primed with either oligo$(dT)_{18}$ primer, random primers or gene-specific primers.
Oligo$(dT)_{18}$ primes cDNA synthesis from the poly(A) tail present at the 3'-end of eukaryotic mRNA. Random primers initiate cDNA synthesis from the total RNA population (rRNA and mRNA). Therefore, using random primers for first strand synthesis results in a greater complexity of the generated cDNA compared with the oligo$(dT)_{18}$ primer. As a consequence, the sensitivity and specificity of subsequent PCR reactions may be reduced. However, there are several applications where it is beneficial to use random primers, such as cDNA synthesis using mRNAs without a poly(A) tail, or cDNA synthesis using poly(A)-enriched RNA samples.
Gene-specific primers are used to synthesize specific cDNA from a pool of total RNA or mRNA and must be obtained by the user.

Control Reactions for RT-PCR / RT-qPCR

The following negative control reactions should be used to verify the results of the first strand cDNA synthesis.

- **Reverse transcriptase minus (RT-) negative control** is important in RT-PCR and RT-qPCR reactions to assess for genomic DNA contamination of the RNA sample. The control RT- reaction should contain all reagents for the reverse transcription reaction except the Maxima H Minus Enzyme Mix.
- **No template control (NTC)** is important to assess for reagent contamination. The NTC reaction should contain all reagents necessary for the reverse transcription reaction except the RNA template.

Protocols

Please read the IMPORTANT NOTES section (page 2) before starting.

RT-PCR

I. First Strand cDNA Synthesis

After thawing, mix and briefly centrifuge the components of the kit. Store on ice.

1. Add the following reagents into a sterile, RNase free tube on ice in the indicated order:

Component	Volume
Template RNA total RNA *or* poly(A) mRNA *or* specific RNA	 1 pg - 5 µg 0.1 pg – 500 ng 0.01 pg - 500 ng
Primers oligo $(dT)_{18}$ primer *or* random hexamer primer *or* gene-specific primer	 1 µL (100 pmol) 1 µL (100 pmol) 2-20 pmol
10 mM dNTP Mix	1 µL (0.5 mM final concentration)
Water, nuclease-free	to 15 µL

2. *Optional.* If the RNA template is GC-rich or is known to contain secondary structures, mix gently, centrifuge briefly and incubate at 65 °C for 5 min. Chill on ice, briefly centrifuge again and place on ice.
3. Add the following components in the indicated order:

Component	Volume
5X RT Buffer	4 µL
Maxima H Minus Enzyme Mix	1 µL
Total volume	20 µL

Mix gently and centrifuge. Incubate: – if an oligo$(dT)_{18}$ primer or gene-specific primer is used, incubate for 30 min at 50 °C.
– if a random hexamer primer is used, incubate for 10 min at 25 °C followed by 30 min at 50 °C.
– for transcription of GC-rich RNA, the reaction temperature can be increased to 65 °C.

4. Terminate the reaction by heating at 85 °C for 5 minutes.

The reaction product of the first strand cDNA synthesis can be used directly in PCR or stored at -20 °C for up to one week. For longer storage, -70 °C is recommended. Avoid freeze/thaw cycles of the cDNA.

II. PCR

The product of the first strand cDNA synthesis reaction can be used directly in PCR. The volume of the first strand cDNA synthesis reaction mixture should not comprise more than 1/10 of the total PCR reaction volume. Normally, 2 µL of the first strand cDNA synthesis reaction mixture is used as template for subsequent PCR in a 25 µL total volume. Thermo Scientific™ DreamTaq™ DNA polymerase is suitable for amplification of fragments up to 6 kb. Thermo Scientific™ Phusion™ High-Fidelity DNA Polymerases are recommended to generate amplicons up to 20 kb.

RT-qPCR

I. First Strand cDNA Synthesis

The following protocol is optimized to generate first-strand cDNA for use in two-step RT-qPCR.
After thawing, mix and briefly centrifuge the components of the kit. Store on ice.

1. Add the following reagents into a sterile, RNase free tube on ice in the indicated order:

Component	Volume
Template RNA total RNA *or* poly(A) mRNA *or* specific RNA	 1 pg - 5 µg 0.1 pg – 500 ng 0.01 pg - 500 ng
Primers oligo $(dT)_{18}$ primer *or* random hexamer primer *or* gene-specific primer	 0.25 µL (25 pmol) 0.25 µL (25 pmol) 2-20 pmol
10 mM dNTP Mix	1 µL
Water, nuclease-free	to 15 µL

2. *Optional.* If the RNA template is GC-rich or is known to contain secondary structures, mix gently, centrifuge briefly and incubate at 65 °C for 5 min. Chill on ice, briefly centrifuge again and place on ice.
3. Add the following components to the reaction tube in the indicated order:

Component	Volume
5X RT Buffer	4 µL
Maxima H Minus Enzyme Mix	1 µL
Total volume	20 µL

Mix gently and centrifuge.

4. Incubate for 10 min at 25°C followed by 15 min at 50 °C.
5. Terminate the reaction by heating at 85 °C for 5 minutes.

Note. For RNA template quantities greater than 1 µg, prolong the reaction time to 30 min. For RNA templates that are GC-rich or have a large amount of secondary structure, the reaction temperature can be increased to 65 °C.
The reverse transcription reaction product can be used directly in qPCR or stored at -20 °C for up to one week. For longer storage, -70 °C is recommended. Avoid freeze/thaw cycles of the cDNA.

II. qPCR

The product of the first strand cDNA synthesis reaction can be used directly in qPCR. The volume of first strand cDNA synthesis reaction mixture should not comprise more than 1/10 of the total PCR reaction volume. Normally, 2 µL of the RT mixture is used as template for subsequent qPCR in 25 µL total volume.

Troubleshooting

Low yield or no product in RT-PCR or RT-qPCR
Poor integrity of RNA template. RNA purity and integrity is essential for synthesis and quantification of cDNA. Always assess the integrity of RNA prior to cDNA synthesis Use freshly prepared RNA. Multiple freeze/thaw cycles of the RNA sample and synthesized cDNA is not recommended. Follow general recommendations to avoid RNase contamination and discard low quality RNA. **Low template purity (inhibitors in RNA sample).** Trace amounts of agents used in RNA purification protocols may remain in solution and inhibit first strand synthesis, e.g., SDS, EDTA, guanidine salts, phosphate, pyrophosphate, polyamines, spermidine. To remove trace contaminants, re-precipitate the RNA with ethanol and wash the pellet with 75 % ethanol. **Insufficient template quantity.** Increase the amount of RNA template in the first strand reaction to the recommended level. Following DNase I treatment, terminate the reaction by heat inactivation of the enzyme in the presence of EDTA (to bind Mg^{2+}). **GC rich template.** If the RNA template is GC rich or is known to contain secondary structures, the temperature of the reverse transcription reaction can be increased up to 65 °C. **Excess amount of cDNA in qPCR/PCR.** Decrease amount of cDNA synthesis reaction in qPCR/PCR. The volume of the cDNA synthesis reaction mixture should not exceed 10 % of the final PCR reaction mixture.
No product in long RT-PCR (>5 kb)
Suboptimal priming. Use oligo $(dT)_{18}$ primer or gene specific primer. If random primers are used, reduce the amount of random primers to 25 pmol per 20 µL reaction mixture.
RT-PCR product longer than expected
RNA template is contaminated with DNA. Amplification of genomic DNA containing introns. To avoid amplification of genomic DNA, design PCR primers on exon-exon boundaries or perform DNase I digestion prior reverse transcription.
RT-PCR product in RT-minus control
RNA template is contaminated with DNA. The presence of a PCR product in the negative control (RT-) reaction indicates that the reaction is contaminated with DNA. To avoid amplification of genomic DNA, design PCR primers on exon-exon boundaries or perform DNase I digestion prior reverse transcription.

References

1. Wiame, I., et al., Irreversible heat inactivation of DNaseI without RNA degradation, BioTechniques, 29, 252-256, 2000.
2. Fleige, S., Pfaffl, M.W., RNA integrity and the effect on the real-time qRT-PCR performance, Mol. Aspects Med., 27, 126-139, 2006.
3. Nolan, T., et al., Quantification of mRNA using real-time RT-PCR, Nat. Protoc., 1, 1559-1582, 2006.

Revision history: Pub. No. MAN0012720

Revision	Date	Description
B00	2024-02-26	Revized user guide template, removed COA content and updated related products

Limited product warranty

Life Technologies Corporation and/or it affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html.** If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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