Maxima First Strand cDNA Synthesis Kit for RT-qPCR with dsDNase

Catalog Number K1671, K1672

Pub. No. MAN0012951 **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
K1671 (50 rxns)	Maxima Enzyme Mix	100 µL	-25 °C to -15 °C
	5X Reaction Mix	200 µL	
	dsDNase	50 µL	
	10X dsDNase Buffer	100 µL	
	Water, nuclease-free	1.25 mL	
K1672 (200 rxns)	Maxima Enzyme Mix	4 x 100 µL	
	5X Reaction Mix	800 µL	
	dsDNase	4 x 50 µL	
	10X dsDNase Buffer	2 x 100 µL	
	Water, nuclease-free	4 x 1.25 mL	

Description

Thermo Scientific™ Maxima™ First Strand cDNA Synthesis Kit for RT-qPCR with dsDNase provides a simplified workflow that combines genomic DNA elimination and cDNA synthesis into one-tube procedure. It is a convenient system optimized for cDNA synthesis in two-step real-time quantitative RT-PCR (RT-qPCR) applications. The kit contains a novel double-strand specific DNase (dsDNase) engineered to remove contaminating genomic DNA from RNA preps in 2 minutes without damage to quality or quantity of RNA. Highly specific dsDNase activity towards double-stranded DNA ensures that RNA and single-stranded DNA (such as cDNA and primers) are not cleaved and dsDNase treated RNA can be directly added to reverse transcription. The kit uses Maxima Enzyme Mix containing Maxima Reverse Transcriptase (RT) and Thermo Scientific™ RiboLock™ RNase Inhibitor. Maxima Reverse Transcriptase is an advanced enzyme derived by *in vitro* evolution of M-MuLV RT. The enzyme features high thermostability, robustness and increased cDNA synthesis rate compared to wild type M-MuLV RT. The recombinant RiboLock RNase Inhibitor effectively protects RNA template from degradation by RNases A, B and C at temperatures up to 55 °C. 5X Reaction Mix contains the remaining reaction components: reaction buffer, dNTPs, oligo $(dT)_{18}$ and random hexamer primers.

The Maxima First Strand cDNA Synthesis Kit is capable of reproducible cDNA synthesis from a wide range of starting total RNA amounts (1 pg - 5 µg) at elevated temperatures (50-65 °C). The synthesis reaction can be completed in 15-30 minutes.

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.
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.

Primers

The Maxima First Strand cDNA Synthesis Kit contains oligo$(dT)_{18}$ and random hexamer primers to prime synthesis of first strand cDNA. This primer mixture ensures high sensitivity in low copy number transcript detection assays.

Protocol

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
10X dsDNase Buffer	1 µL
dsDNase	1 µL
Template RNA	1 pg – 5 µg total RNA *or* 0.1 pg – 500 ng poly(A) mRNA *or* 0.01 pg – 500 ng specific RNA
Water, nuclease-free	to 10 µL
Total volume	10 µL

2. Mix gently and centrifuge.
3. Incubate for 2 min at 37 °C in preheated thermomixer or water bath. Chill on ice, briefly centrifuge and place on ice.
4. Add the following components to the same tube:

Component	Volume
5X Reaction Mix	4 µL
Maxima Enzyme Mix	2 µL
Water, nuclease-free	4 µL

5. Mix gently and centrifuge.
6. Incubate for 10 min at 25 °C followed by 15 min at 50 °C.

Note. For RNA template amounts 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.

7. 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 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.

qPCR

The product of the first strand cDNA synthesis reaction can be used directly in qPCR. Normally, 2 µL of the RT mixture is used as template for subsequent qPCR in a 25 µL total volume.

Control Reactions

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 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.

Troubleshooting

No qPCR product generated or product appears late in 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. **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.** Decrease amount of cDNA synthesis reaction in qPCR. The volume of the cDNA synthesis reaction mixture should not exceed 10 % of the final PCR reaction mixture.
RT-qPCR 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. Follow the protocol carefully and make sure that dsDNase treatment step prior reverse transcription is not omitted. To further enhance genomic DNA elimination efficiency, template RNA incubation with dsDNase step can be prolonged to 5 minutes.

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. MAN0012951

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

Limited product warranty

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