thermo scientific

PRODUCT INFORMATION

Thermo Scientific Maxima™ H Minus cDNA Synthesis Master Mix with dsDNase

Pub. No. MAN0016393
Rev. Date 9 January 2017 (Rev. B.0)

Kit contents

Component	#M1669 (10 rxns)	#M1681 (50 rxns)	#M1682 (200 rxns)
Maxima H Minus cDNA Synthesis Master Mix (5X)	40 µL	200 µL	800 µL
Maxima No RT Control	40 µL	200 µL	200 µL
dsDNase	10 µL	50 µL	200 µL
10X dsDNase Buffer	20 µL	100 µL	400 µL
Water, nuclease-free	1.25 mL	1.25 mL	1.25 mL

Store at –20°C

www.thermofisher.com

DESCRIPTION

The Thermo Scientific™ Maxima™ H Minus cDNA Synthesis Master Mix with dsDNase provides a simple workflow that combines genomic DNA elimination and cDNA synthesis in a one-tube procedure. The system is convenient, and optimized for cDNA synthesis in two-step real time quantitative RT-PCR (RT-qPCR) applications.

The kit contains a double-strand specific DNase (dsDNase) engineered to remove contaminating genomic DNA from RNA samples in 2 minutes without affecting 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 allows dsDNase treated RNA to be directly added to reverse transcription reactions.

The master mix contains Maxima H Minus Reverse Transcriptase (RT) and Thermo Scientific™ RiboLock™ RNase Inhibitor. Maxima H Minus Reverse Transcriptase is an advanced enzyme derived from 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. The master mix also contains reaction buffer, dNTPs, oligo $(dT)_{18}$, and random hexamer primers.

The Maxima H Minus cDNA Synthesis Master Mix is capable of reproducible cDNA synthesis starting with 1 pg to 5 µg of total RNA at elevated temperatures (50–65°C). The synthesis reaction can be completed in 15–30 minutes.

PROCEDURAL GUIDELINES

Avoiding ribonuclease contamination

RNA purity and integrity is essential for synthesis of full-length cDNA. RNA can be degraded by RNase A, which is a highly stable contaminant found in laboratory environments. 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 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

The kit is compatible with total cellular RNA isolated by standard methods. Purified RNA must be free of salts, metal ions, ethanol, and phenol to avoid inhibiting the cDNA synthesis reaction.

Always perform a No RT Control reaction, which includes all components for reverse transcription except the Maxima H Minus RT.

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 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. Smearing of rRNA bands is an indication of degraded mRNA, and a new sample of total RNA should be prepared.

Alternatively, total RNA can be analyzed using a bioanalyzer (e.g., Agilent 2100) to obtain a RNA integrity number, which provides quantitative information about the general state of the RNA sample (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 H Minus cDNA Synthesis Master Mix 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

Allow kit components to thaw, then mix and briefly centrifuge. Store the tubes on ice.

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

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

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

Maxima cDNA H Minus Synthesis Master Mix (5X)	4 µL
Water, nuclease-free	6 µL

6. Mix gently and centrifuge.
7. Incubate at 25°C for 10 minutes.
8. Incubate at 50°C for 15 minutes.
   Note. If using >1 µg RNA template, increase the reaction time to 30 minutes. For RNA templates that are GC-rich or have a large amount of secondary structure, the reaction temperature can be increased to 65°C.
9. 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. Store at –20°C for up to one week, or –70°C for long term storage. Avoid freeze-thaw cycles of the cDNA.

qPCR

Use the product of the cDNA synthesis reaction directly in qPCR. Normally, 2 µL of the RT product is used as template for subsequent qPCR in a 25 µL total volume.

CONTROL REACTIONS

Use the following negative control reactions to verify the results of the first strand cDNA synthesis.

- **No RT control** to assess genomic DNA contamination of the RNA sample. The Maxima No RT Control reaction includes all reagents for the reverse transcription reaction except the Maxima H Minus RT.
- **No template control** (NTC) to assess for reagent contamination. The NTC reaction includes all reagents necessary for the reverse transcription reaction except the RNA template.

(continued on reverse page)

Prepare control reactions according to the following instructions.

Note: Template RNA amount and reverse transcription incubation conditions should reflect the conditions used for experimental samples.

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

Component	No RT control	No template control
Maxima H Minus cDNA Synthesis Master Mix (5X)	—	4 µL
Template RNA	varies	—
Water, nuclease-free	to 20 µL	to 20 µL
Total volume	20 µL	20 µL

2. Mix gently and centrifuge.
3. Incubate at 25°C for 10 minutes.
4. Incubate at 50°C for 15 minutes.
5. Terminate the reaction by heating at 85°C for 5 minutes.

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).

Remove trace contaminants by re-precipitating 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 No RT Control

RNA template is contaminated with DNA.

The presence of a PCR product in the negative control (No RT Control) 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.

IMPORTANT LICENSING INFORMATION

These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Refer to **www.thermofisher.com/support** for the Safety Data Sheets.