PRODUCT INFORMATION

Thermo Scientific Maxima H Minus First Strand cDNA Synthesis Kit with dsDNase

#K1682 100 rxns

Lot __ Expiry Date __

Store at -20°C

www.thermoscientific.com/onebio

COMPONENTS OF THE KIT

Maxima H Minus First Strand cDNA Synthesis Kit with dsDNase	#K1681 20 rxns	#K1682 100 rxns
Maxima H Minus Enzyme Mix	25 µL	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	150 µL	500 µL
10 mM dNTP Mix	50 µL	250 µL
Oligo$(dT)_{18}$ Primer, 100 µM	25 µL	120 µL
Random Hexamer Primer, 100 µM	25 µL	120 µL
dsDNase	20 µL	2 x 50 µL
10X dsDNase Buffer	100 µL	100 µL
Water, nuclease-free	1.25 mL	2 × 1.25 mL

CERTIFICATE OF ANALYSIS

Functional tests

RT-PCR using 100 fg of control GAPDH RNA and GAPDH control primers generated a prominent 496 bp product on 1% agarose gel after ethidium bromide staining.

dsDNase is functionally tested in removal of genomic DNA contamination from RNA sample and subsequent RT-qPCR amplification. Efficiency of genomic DNA removal is ≥99.85%. RNA quantity is not affected by dsDNase treatment.

Quality authorized by: Jurgita Zilinskiene

Rev.1

DESCRIPTION

Thermo Scientific™ Maxima™ H Minus First Strand cDNA Synthesis Kit with dsDNase provides a dramatically simplified workflow that combines genomic DNA elimination and cDNA synthesis into one-tube procedure. 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 the Maxima H Minus Reverse Transcriptase (RT), an advanced enzyme derived by in vitro evolution of M-MuLV RT. The enzyme features the highest thermostability among the derivatives of M-MuLV RT 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 (50-65 °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 with a variety of thermostabile DNA polymerases, in qPCR with Thermo Scientific Maxima qPCR Master Mixes 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.

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.

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PROTOCOLS

Please read the IMPORTANT NOTES section before starting. After thawing, mix and briefly centrifuge the components of the kit. Store on ice.

Genomic DNA elimination

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

10X dsDNase Buffer		1 µL
dsDNase		1 µL
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
Water, nuclease-free		To 10 µL

2. Mix gently and spin down.
3. Incubate for 2 min at 37°C in the preheated thermomixer or water bath.
4. *Optional.* If If RNA sample is to be used for RT-PCR amplification of long fragments (≥ 3 kb), perform dsDNase inactivation by incubating the sample at 55°C for 5 min in the presence of 10 mM DTT.
5. Chill on ice, briefly centrifuge again and place on ice.
6. Add first strand cDNA synthesis reagent to the same tube according the following RT-PCR or RT-qPCR protocols.

RT-PCR

I. First Strand cDNA Synthesis.

The following protocol is optimized to generate first-strand cDNA for use in two-step RT-qPCR.

1. After genomic DNA elimination, add the following reagents into the same tube in the indicated order:

Primer	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:

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. *Taq* DNA polymerase, PCR Master Mix (2X) or Maxima Hot Start PCR Master Mix (2X) can be used to amplify fragments less than 3 kb. Thermo Scientific™ DreamTaq™ DNA polymerase is suitable for amplification of longer 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.

1. After genomic DNA elimination, add the following reagents into the same tube in the indicated order:

Primers	oligo $(dT)_{18}$ primer and 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:

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. Maxima H Minus First Strand cDNA Synthesis Kit is optimized for use with Thermo Scientific™Luminaris™ qPCR master mixes.

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.

Note:

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Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.

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 reagents 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 in RT-minus control

RNA template is contaminated with DNA.
The appearance of a PCR product in the negative control reaction (RT-) indicates that the reaction is still contaminated with DNA. To completely remove residual DNA, prolong RNA incubation with dsDNase step up to 5 minutes.
Note that trace amounts of reagents used in RNA purification protocols (e.g., SDS, EDTA, guanidine salts, phosphate, pyrophosphate, polyamines, spermidine) may remain in solution and inhibit HL-dsDNase activity. To remove trace contaminants, re-precipitate the RNA with ethanol, wash the pellet with 75% ethanol and dissolve in nuclease-free water.