PRODUCT INFORMATION

Thermo Scientific
Maxima First Strand cDNA Synthesis Kit for RT-qPCR

#K1641 for 50 rxns

Lot __
Expiry date __

Store at -20°C

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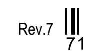
CERTIFICATE OF ANALYSIS

Functionally tested in two-step RT-qPCR using different starting amounts (5 ng – 0.5 fg) of RNA transcript in reverse transcription reactions followed by amplification with Thermo Scientific Maxima SYBR Green/ROX qPCR Master Mix. Reaction efficiency is in the range of 90-110%, slope is between -3.09 and -3.58 with a correlation coefficient of >0.99.

Quality authorized by: Jurgita Zilinskiene



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COMPONENTS OF THE KIT

Maxima First Strand cDNA Synthesis Kit for RT-qPCR	#K1641 50 rxns	#K1642 200 rxns
Maxima Enzyme Mix	100 µL	400 µL
5X Reaction Mix	200 µL	800 µL
Water, nuclease-free	1.25 mL	4 × 1.25 mL

STORAGE

All kit components should be stored at -20°C.

DESCRIPTION

The Maxima® First Strand cDNA Synthesis Kit is a convenient system optimized for cDNA synthesis in two step real time quantitative RT-PCR (RT-qPCR) applications. The kit uses Maxima Reverse Transcriptase, 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 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.
Components of the Maxima First Strand cDNA Synthesis Kit are premixed to save time and to reduce the possibility of pipeting errors. The contents of the kit include the Maxima Enzyme Mix, 5X Reaction Mix and Water, nuclease-free.

Maxima Enzyme Mix contains Maxima Reverse Transcriptase and Thermo Scientific RiboLock RNase Inhibitor. 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.

Water, nuclease-free is provided for reaction set-up and dilution of sample RNA. The absence of endo-, exodeoxyribonucleases, ribonucleases and phosphatases has been confirmed by appropriate quality tests.

IMPORTANT NOTES

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 any laboratory environment. All components of the kit have been rigorously tested to ensure that they are RNase free. 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 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-qPCR applications, template RNA must be free of DNA contamination. Prior to cDNA synthesis, RNA can be treated with DNase I, RNase-free (#EN0521) to remove residual DNA.
Always perform an RT-minus negative control reaction that includes all the components for the RT reaction except for the Maxima 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. 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 FOR RT-qPCR

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

First Strand cDNA Synthesis

The first strand cDNA synthesis reaction can be performed as an individual reaction or as a series of parallel reactions with different RNA templates. Therefore, the reaction mixture can be prepared by combining reagents individually or a master mix containing all of the components for the RT reaction except template RNA.
After thawing, mix and briefly centrifuge the components of the kit. Store on ice.

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

5X Reaction Mix	4 µL
Maxima Enzyme Mix	2 µL
Template RNA	1 pg - 5 µg
Water, nuclease-free	to 20 µL
Total volume	20 µL

2 Mix gently and centrifuge.
3. 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.
4. Terminate the reaction by heating at 85°C for 5 min.

The 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 cDNA.

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 a 25 µL total volume. The Maxima First Strand cDNA Synthesis Kit is optimized for use with Maxima qPCR master mixes (#K0221, #K0231, #K0241, K0251, #K0261).

CONTROL REACTIONS

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-qPCR reactions to assess for genomic DNA contamination of the RNA sample. The control RT- reaction should contain every reagent for the reverse transcription reaction except for the Maxima Enzyme Mix.
- **No template control (NTC)** is important to assess for reagent contamination. The NTC reaction should contain all reagents for the reverse transcription reaction except for the RNA template.

TROUBLESHOOTING

Problem	Cause and Solution
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. (*see* page 2). Follow general recommendations to avoid RNase contamination (page 2) and discard low quality RNA. Use freshly prepared RNA. Multiple freeze/thaw cycles of the RNA sample as well as synthesized cDNA is not recommended. **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 (*see* Protocol for RT-qPCR on p.3). Following DNase I treatment, terminate the reaction by heat inactivation of the enzyme in the presence of EDTA (to bind Mg^{2+}). RNA hydrolyzes during heating in the absence of a chelating agent (1). **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 the amount of the cDNA synthesis reaction mixture added to the qPCR reaction. The volume of the cDNA synthesis reaction mixture should not exceed 10% of the final volume of the qPCR reaction mixture.
RT-qPCR 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 contaminated with DNA. To avoid amplification of genomic DNA, design PCR primers on exon-intron 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.

Note:
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