Maxima reverse transcriptases

Thermo Scientific™ Maxima™ reverse transcriptases (RTs) are available as stand-alone enzymes, as components in the Thermo Scientific™ Maxima™ First Strand cDNA Synthesis Kit, or in master mix formats, as in the Thermo Scientific™ Maxima™ H Minus cDNA Synthesis Master Mix for first-strand cDNA synthesis in RT-PCR and RT-qPCR.

General guidelines for cDNA synthesis

- Use freshly prepared RNA. Check the integrity of RNA prior to cDNA synthesis, by agarose gel electrophoresis or using the Agilent™ 2100 Bioanalyzer™ instrument.
- For RT-qPCR applications, template RNA must be free of DNA contamination. Remove residual genomic DNA (gDNA) from the RNA prep prior to qPCR to ensure reliable quantification. Use a no-RT control to assess gDNA contamination of the RNA sample.

Choose the appropriate format for your application and usage

Format	Components	Usage
Enzyme only	Maxima H Minus RT enzyme	Maximum flexibility in reaction setup
	5X RT buffer	
Kit	Maxima H Minus RT Enzyme Mix	Versatile, includes all components for the first-strand cDNA synthesis reaction
	Oligo(dT)$_{18}$ and random hexamers	
	5X RT buffer	
	dNTP mix	
	Nuclease-free water	
Master mix	Maxima H Minus cDNA Synthesis Master Mix	Maximum convenience, helps minimize cDNA synthesis variability, complete solution
	Maxima H Minus cDNA Synthesis Master Mix, no-RT control	
	Nuclease-free water	

* Maxima RT kits and master mixes are also available with and without Thermo Scientific™ double-stranded DNA–specific DNase (Thermo Scientific™ dsDNase).

First-strand cDNA synthesis

Stand-alone enzyme or Maxima First Strand cDNA Synthesis Kit

Mix and briefly centrifuge all reagents after thawing, keep on ice, and follow the steps:

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

1	**Template RNA—choose one**	
	• Total RNA	1 pg–5 µg
	• Poly(A) mRNA	0.1 pg–500 ng
	• Specific RNA	0.01 pg–500 ng
2	**Primer—choose one**	
	• Oligo(dT)$_{18}$	1 µL (100 pmol)
	• Random hexamer	1 µL (100 pmol)
	• Gene-specific primer	12–20 pmol
3	Thermo Scientific™ dNTP Mix (10 mM each)	1 µL (0.5 mM final concentration)
4	Water, nuclease-free	To 14.5 µL
	Total volume	14.5 µL

2. Optional: For GC-rich RNA or RNA templates with secondary structures, mix gently, centrifuge briefly, and incubate at 65°C for 5 min. Chill on ice, briefly centrifuge again, and place back on ice.

3. Add the following reaction components in the indicated order:

1	5X RT buffer	4 µL
2	Thermo Scientific™ RiboLock™ RNase Inhibitor	0.5 µL (20 U)
3	Maxima H Minus RT	50–200 U
	Water, nuclease-free	To 20 µL
	Total volume	20 µL

4. Mix gently and centrifuge briefly.

5. Incubate for the specified times depending on the primer used:
   - Oligo(dT)$_{18}$ primer or gene-specific primer: 15–30 min at 50°C
   - Random hexamer primer: 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.

6. Terminate the reaction by heating at 85°C for 5 min.

Note: To generate the highest amounts of reverse transcription reaction products (in applications such as synthesis of labeling probes), use 200 U of enzyme per reaction. For downstream applications, such as PCR or qPCR, optimize enzyme amounts within a range of 50–200 U.

Maxima H Minus cDNA Synthesis Master Mix

Allow kit components to thaw, then mix and briefly centrifuge, store the tubes on ice, and follow the steps below:

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

   If you are not using dsDNase for gDNA removal, take the appropriate amount of input template RNA indicated below and proceed to step 5.

1	10X dsDNase buffer	1 µL
2	dsDNase	1 µL
3	**Template RNA—choose one** • Total RNA • Poly(A) mRNA • Specific RNA	 1 pg–5 µg 0.1 pg–500 ng 0.01 pg–500 ng
4	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 min.
4. Place on ice, briefly centrifuge, and place it back on ice.
5. Add the following components to the tube on ice in the order below:

1	Maxima H Minus cDNA Synthesis Master Mix (5X)	4 µL
2	**Template RNA—choose one** • Total RNA • Poly(A) mRNA • Specific RNA	 1 pg–5 µg 0.1 pg–500 ng 0.01 pg–500 ng
3	Water, nuclease-free	To 20 µL
	Total volume	20 µL

6. Mix gently and centrifuge.
7. Incubate at 25°C for 10 min.
8. Incubate at 50°C for 15 min.

Note: If using >1 µg RNA template, increase 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.

9. Terminate the reaction by heating at 85°C for 5 min.

Notes for downstream qPCR application

- The reaction product of the first-strand cDNA synthesis can be used directly in qPCR. Store at –20°C for up to 1 week, or at –70°C for long-term storage. Avoid freeze-thaw cycles of the cDNA.
- Use the product of the cDNA synthesis reaction directly in qPCR. Normally, 2 µL of the reverse-transcribed product is used as a template for subsequent qPCR in a total volume of 25 µL.

Related products

- Thermo Scientific™ DNase I, RNase-free enzyme (Cat. No. EN0521)
- dsDNase (Cat. No. EN0771)
- RiboLock RNase Inhibitor (40 U/µL) (Cat. No. EO0381)

Ordering information

Product	Quantity	Cat. No.
Maxima H Minus Reverse Transcriptase	2,000 U/10,000 U/4 x 10,000 U	EP0751/EP0752/EP0753
Maxima H Minus First Strand cDNA Synthesis Kit	20 rxns/100 rxns	K1651/K1652
Maxima H Minus First Strand cDNA Synthesis Kit, with dsDNase	20 rxns/100 rxns	K1681/K1682
Maxima H Minus Double-Stranded cDNA Synthesis Kit	10 rxns	K2561
Maxima H Minus cDNA Synthesis Master Mix	50 rxns/200 rxns	M1661/M1662
Maxima H Minus cDNA Synthesis Master Mix, with dsDNase	50 rxns/200 rxns	M1681/M1682

Find out more at **thermofisher.com/maxima**

ThermoFisher SCIENTIFIC