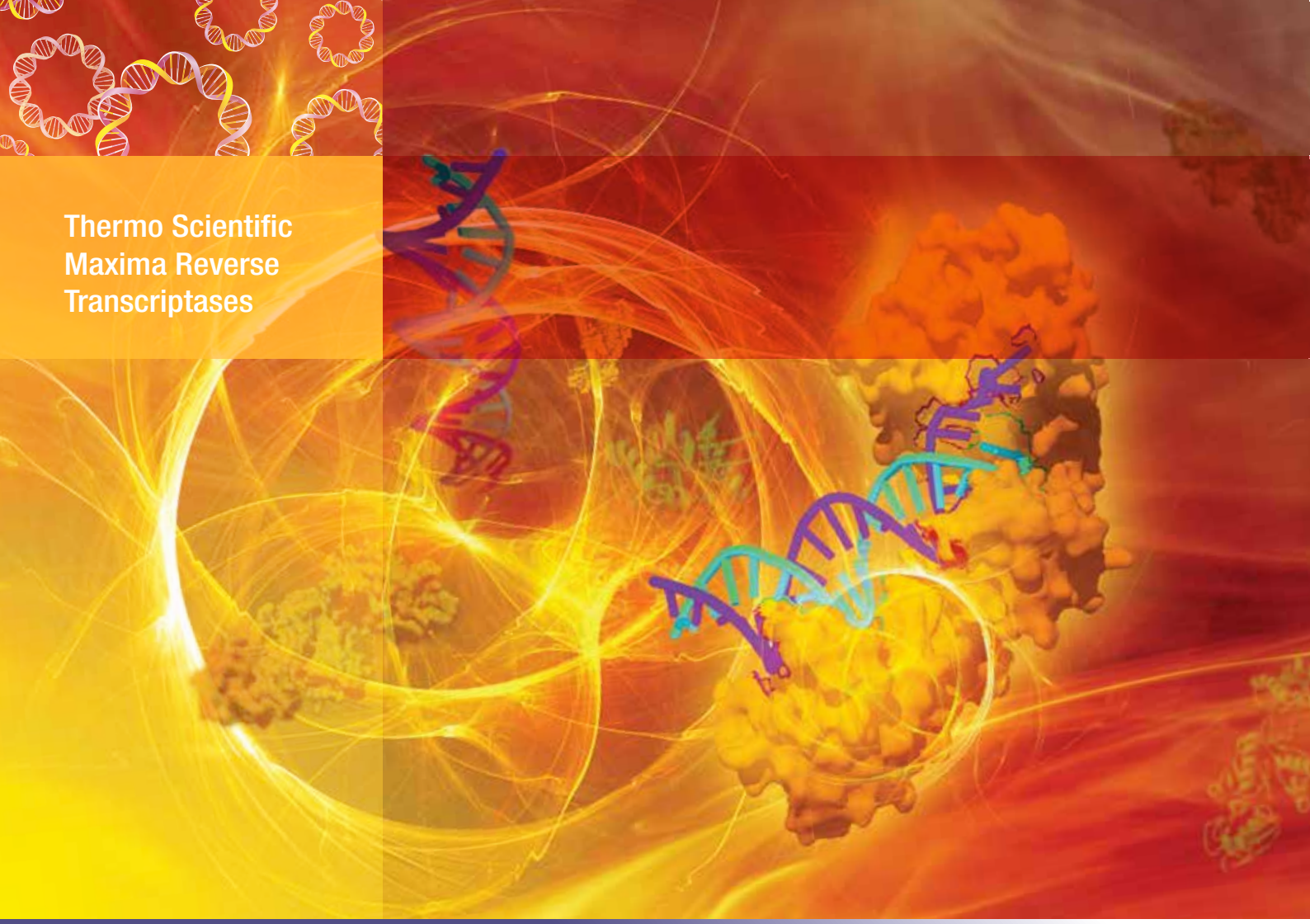


Thermo Scientific
Maxima Reverse
Transcriptases

Molecular evolution

for maximum cDNA

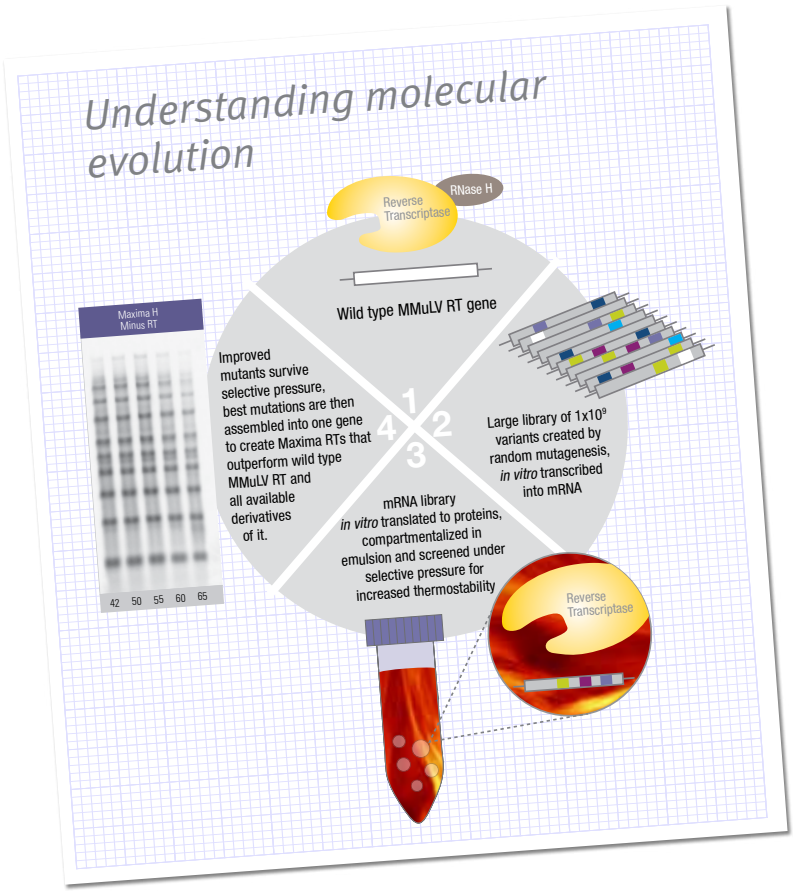
- Maximal reaction temperatures for secondary structure-rich RNA
- Maximal yields of full-length cDNA up to 20 kb for cloning
- Maximal representation of total RNA to eliminate variation in RT-qPCR assays
- Maximal resistance to common reaction inhibitors
- Integrated gDNA removal step

Thermo
SCIENTIFIC

Maximize performance in RT-PCR and RT-qPCR

Thermo Scientific™ Maxima™ Reverse Transcriptases are developed through molecular evolution to maximize performance in cDNA synthesis.

Our proprietary technology for *in vitro* protein evolution has enabled the introduction and selection of multiple favorable mutations into the traditional MMuLV reverse transcriptase[1,2]. This has dramatically improved the enzyme thermostability, robustness and synthesis rates. Maxima Reverse Transcriptase and Maxima H Minus Reverse Transcriptase show 50× increase in processivity and are considerably more resistant to reaction inhibitors such as guanidine and formamide compared to wild type enzymes.

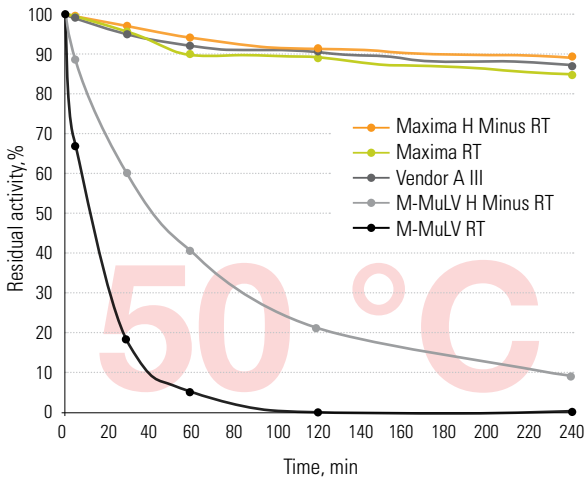


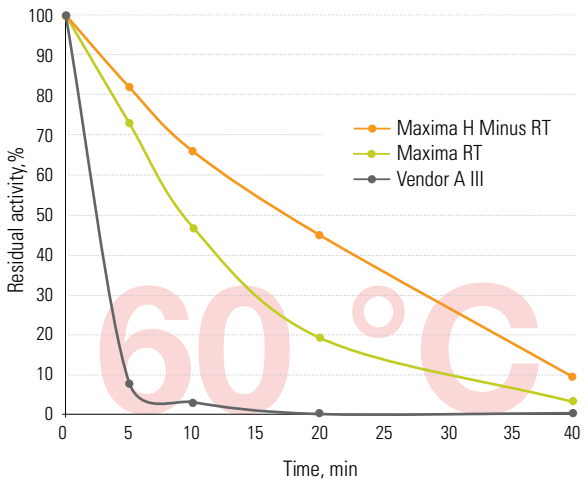
1. Baranauskas *et al.* (2012) "Generation and characterization of new highly thermostable and processive M-MuLV Reverse Transcriptase variants". *PEDS*. doi: 10.1093/protein/gzs034.

2.Janulaitis *et al.* Production of nucleic acid. US patent application US 2011/0065606 A1, 2011 Mar 17.

Very high thermostability for GC-rich templates and durability through reaction

Maxima Reverse Transcriptases feature high thermal stability, leading to improved enzyme performance. cDNA synthesis can be performed at higher temperatures (up to 65 °C) ensuring successful transcription of RNA with high levels of secondary structure. In addition, due to the high thermostability, Maxima Reverse Transcriptases maintain their full activity throughout the RT reaction producing higher cDNA yields.

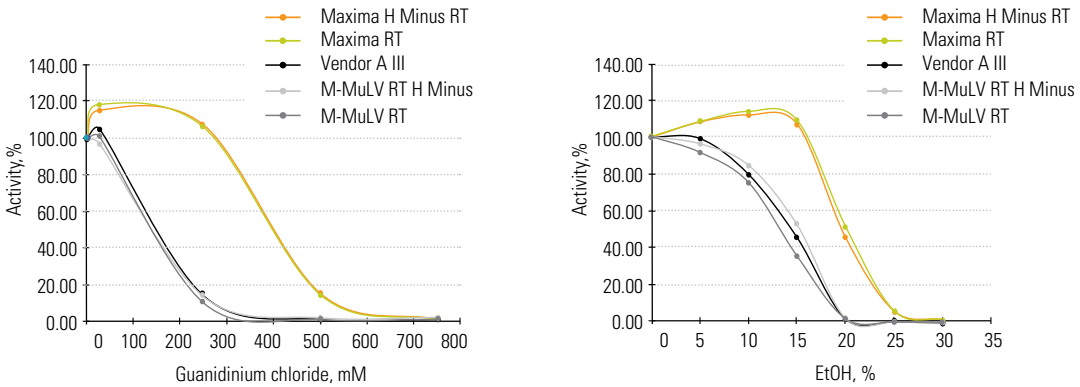




▲ **Residual activity after incubation at elevated temperatures.** RTs were incubated at 50 °C (top) or at 60 °C (bottom) in RT reaction mixture. Residual enzyme activity determined in a standard activity assay at indicated time points.

Reduced effects of reaction inhibitors

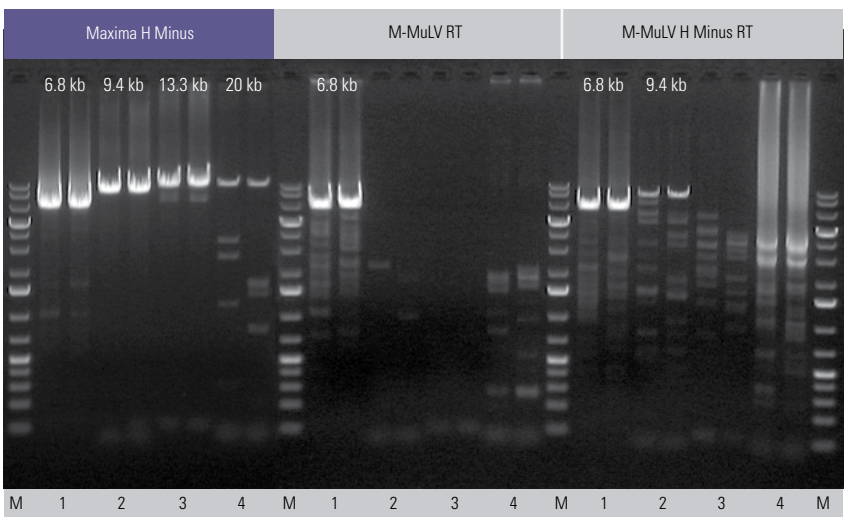
As proven for DNA polymerases such as Thermo Scientific™ Phusion™ DNA Polymerase, increased processivity allows for greater resistance to reaction inhibitors. The same phenomenon can be seen with Maxima Reverse Transciptases. They are 50× more processive than wild-type MMuLV RT and show significantly improved resistance to contaminating inhibitors such as guanidine, ethanol and formamide. This feature helps to obtain prominent results even with lower purity RNA samples such as RNA from FFPE tissues[3].



◀ Maxima H Minus Reverse Transcriptase and Maxima Reverse Transcriptase maintain their activity in the presence of guanidine and ethanol whereas enzyme III from vendor A and wild-type MMuLV RT activity was reduced or lost in the presence of reaction inhibitors.

20 kb RT-PCR product – unrivaled performance in long RT-PCR

Due to its high processivity and lack of RNase H activity, Maxima H Minus Reverse Transcriptase is capable of full-length cDNA synthesis from very long RNA transcripts. As shown below, only Maxima H Minus RT was able to generate 20 kb long RT-PCR product in two-step RT-PCR.



◀ **Amplification of targets up to 20 kb in two-step RT-PCR.** 1 µg of total RNA from Jurkat cells (1 and 2) or total RNA from mouse (3 and 4) were used in a reverse transcription reaction with Maxima H Minus Reverse transcriptase and wild-type RTs in the optimal conditions for each enzyme. Synthesized cDNA was used as a template in PCR with the Long PCR Enzyme Mix (#K0181) and primers specific for different genes:

1 – 6.8 kb POLE (human polymerase)
2 – 9.4 kb FBN1 (human fibrillin 1)
3 – 13.3 kb Dmd (mouse dystrophin)
4 – 20.0 kb Neb (mouse nebulin)
M – Thermo Scientific™ GeneRuler™ 1 kb Plus DNA Ladder (#SM1331)

3. Hagen *et al.* (2013) "A M-MLV reverse transcriptase with reduced RNaseH activity allows greater sensitivity of gene expression detection in formalin fixed and paraffin embedded prostate cancer samples" *Experimental and Molecular Pathology*; 95; 98-104

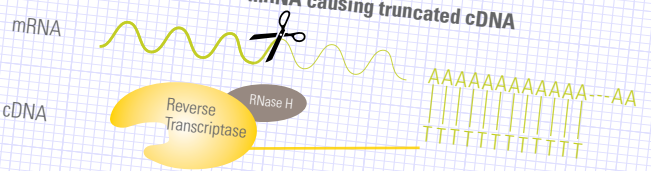
Full-length cDNA at widest temperature range

Maxima Reverse Transcriptase and Maxima H Minus Reverse Transcriptase maintain full activity at widest temperature range from 42 °C to 65 °C superseding all other MMuLV RT derivatives. The high thermostability leads to efficient transcription of RNA regions with high secondary structure and improves specificity when gene-specific primers are used.

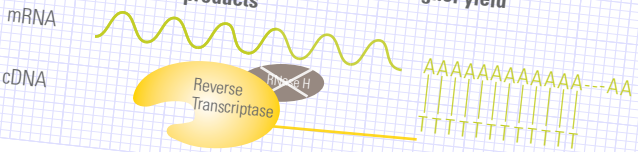
Understanding RNase H activity

Wild type MMuLV RT possesses an RNA-dependent and DNA-dependent polymerase activity as well as RNase H activity. RNase H activity degrades RNA from RNA-DNA duplexes to allow efficient synthesis of dsDNA. However, with long mRNA templates, RNA may be degraded prematurely resulting in truncated cDNA. Hence, it is generally beneficial to minimize RNase H activity when aiming to produce long transcripts for cDNA cloning. In contrast, Reverse Transcriptases with intrinsic RNase H activity are often favored in qPCR applications because they enhance the melting of RNA-DNA duplex during the first cycles of PCR.

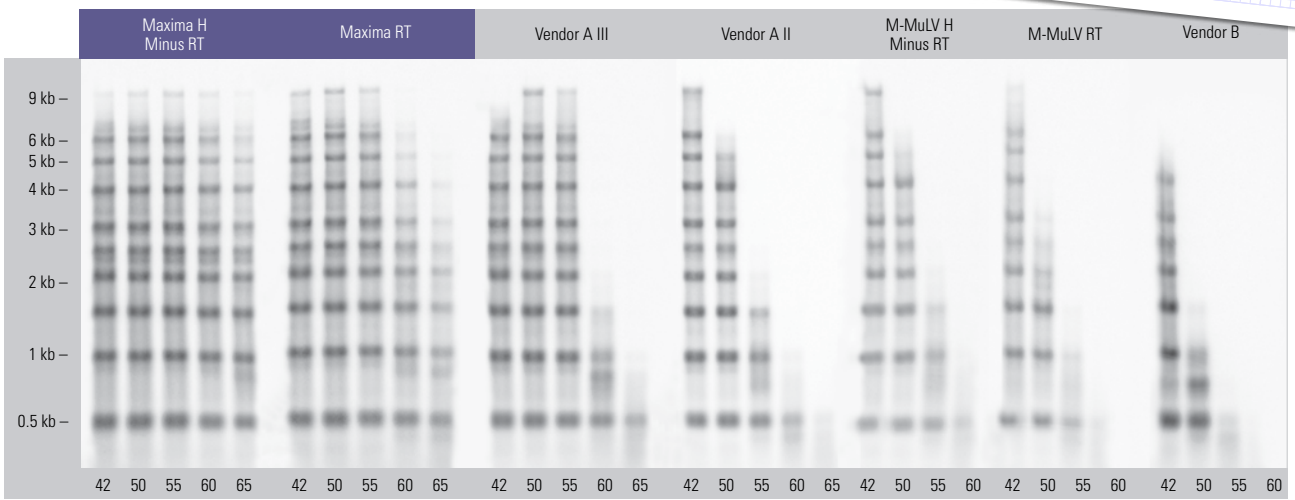
RNase H activity degrades mRNA causing truncated cDNA



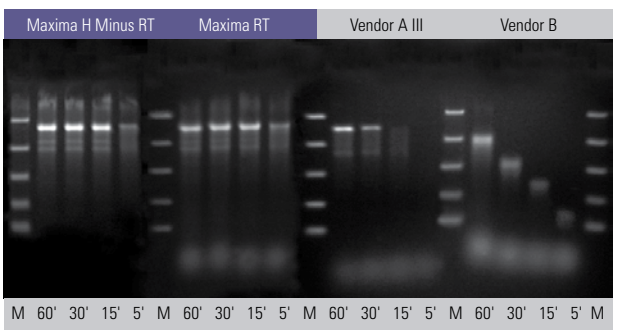
Inactivation of the RNase H activity results in higher yield of full-length cDNA products



High yields of cDNA over a broad temperature range.
cDNA synthesis incorporating radioactive label using 1 µg of Ambion RNA Millennium™ markers (polyA tailed) with oligo(dT)18 primer at different temperatures. Reaction products were resolved on alkaline agarose gel.



7.5 kb cDNA in 5 minutes – fastest RTs in the industry

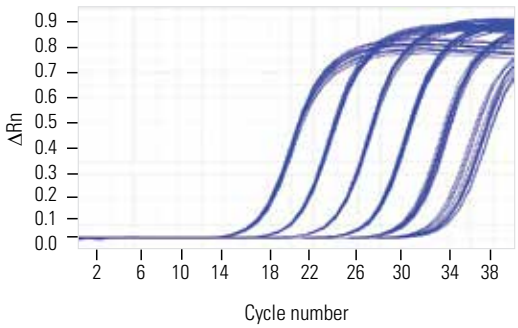


Maxima Reverse Transcriptase and Maxima H Minus Reverse Transcriptase were developed through *in vitro* evolution that resulted in improved enzyme processivity and speed. They are the only RT enzymes capable of synthesizing 7.5 kb long cDNA in just 5 minutes. As shown in the adjacent image, Maxima Reverse Transcriptases allow for the first strand cDNA synthesis reaction to be completed in 5-30 minutes with high yields. In contrast, the RT enzymes from other vendors deliver low or no yields.

High cDNA synthesis rate.
Synthesis of cDNA was performed for 5, 15, 30 and 60 minutes with different RTs at their optimal temperatures using 1 µg of 7.5 kb RNA transcript as a template. Reaction products were analyzed on a 1% alkaline agarose gel.

Ideal for RT-qPCR – sensitive and reproducible quantification

The RT step is one of the greatest sources of variation in RT-qPCR. Ideally, the Cq value should correlate with the initial number of RNA targets. Therefore, the RT enzyme used must perform equally well with a wide range of template amounts. In addition, the RT enzyme should be resistant to the traces of contaminants that arise from the RNA purification step. Maxima Reverse Transcriptase is capable of reproducible cDNA synthesis from 1 pg up to 5 µg in the presence of common reaction inhibitors, which makes it superior choice for your RT-qPCR experiments. To further improve the reproducibility in RT-qPCR, you can select Thermo Scientific™ Maxima™ First Strand cDNA Synthesis Kit with components premixed into two reagent tubes: Maxima RT with ribonuclease inhibitor and the reaction buffer with dNTPs and primers. This composition saves time and reduces variation due to pipetting.



▲

Reproducible cDNA synthesis and low variability levels (<1% SD/Cq) with a wide range of starting RNA amounts. First strand cDNA was generated from 100 ng-1 pg of total Jurkat cell RNA with the Maxima First Strand cDNA Synthesis Kit for RT-qPCR (#K1641) in 16 replicate reactions. Synthesized cDNA was used as a template in subsequent qPCR with Maxima SYBR Green/ROX qPCR Master Mix (#K0221) on the ABI 7500 Real-Time PCR instrument.

RT enzyme selection

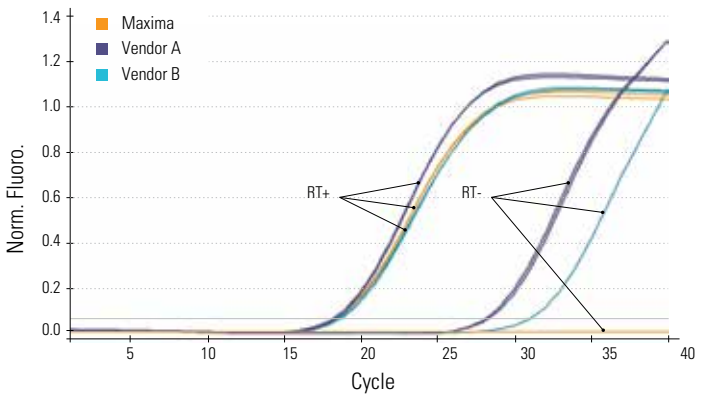
Selection of Thermo Scientific™ Reverse Transcriptases (RTs) include both the advanced enzymes obtained through *in vitro* evolution of M-MuLV RT as well as the wild-type RT enzymes, offering a range of RT enzymes from routine to enhanced performance.

	Enhanced performance		Routine performance	
	Maxima H Minus Reverse Transcriptase	Maxima Reverse Transcriptase	RevertAid H Minus Reverse Transcriptase	RevertAid Reverse Transcriptase
APPLICATIONS				
RT-PCR up to 5 kb	*****	*****	****	****
RT-PCR 5 kb-20 kb	*****	*****	***	**
Cloning	*****	****	***	**
RT-qPCR	****	*****	***	***
cDNA labeling	*****	****	***	**
RACE/Primer extension	*****	**	*****	**1
SPECIFICATIONS				
Optimal reaction temperature	50-55 °C	50-55 °C	42-45 °C	42 °C
Active up to	65 °C	65 °C	55 °C	50 °C
Reading length up to	20 kb	20 kb	13 kb	13 kb
RNase H activity	NO	YES	NO	YES
Sensitivity (total RNA)	1 pg	1 pg	0.1 ng	0.1 ng
Inactivation	85 °C, 5 min	85 °C, 5 min	70 °C, 10 min	70 °C, 10 min
Reaction time	15-30 min	15-30 min	60 min	60 min

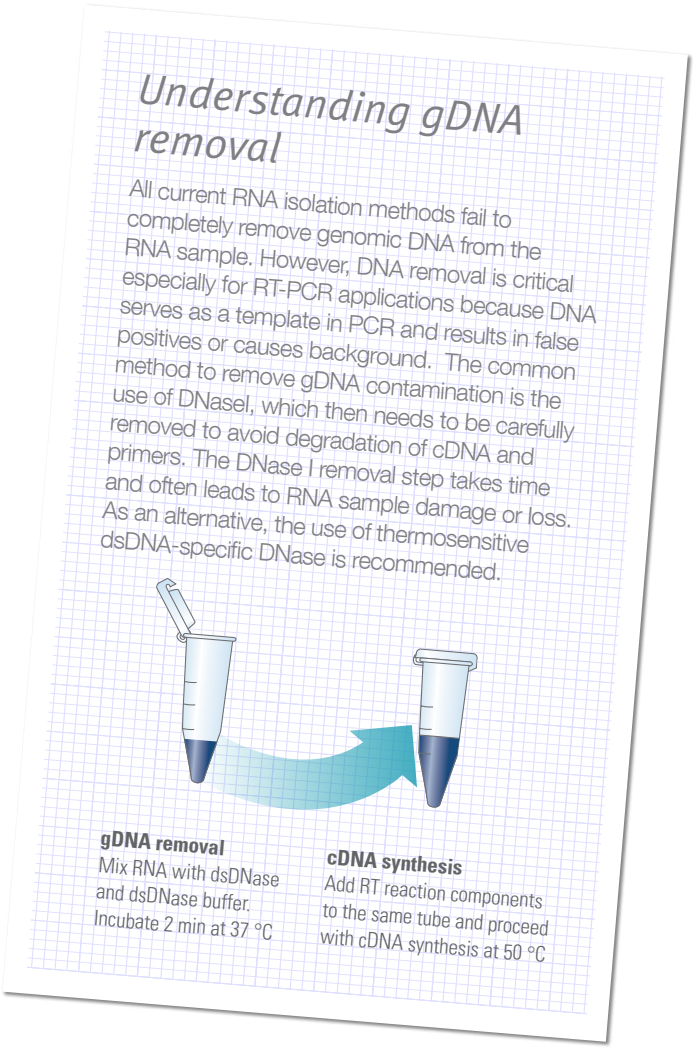
Key: ***** Best performance - * Suitable 1 - not suitable for RACE

NEW Integrated gDNA removal step: novel engineered dsDNase

Our new Maxima and Maxima H Minus First Strand cDNA kits (#K1671, K1681) now include dsDNase to provide a dramatically simplified workflow that combines genomic DNA elimination and cDNA synthesis into one-tube procedure. We use a novel double-strand specific DNase (dsDNase) engineered to remove contaminating genomic DNA from RNA preps in two minutes without damage to quality or quantity of RNA and without damage to single-stranded DNA such as primers and probes. The dsDNase is easily inactivated by modest heat. Using new kit formulations including dsDNase, gDNA is completely and easily eliminated from RNA sample, and there is no need anymore to design your gene expression assays on exon-exon boundaries.



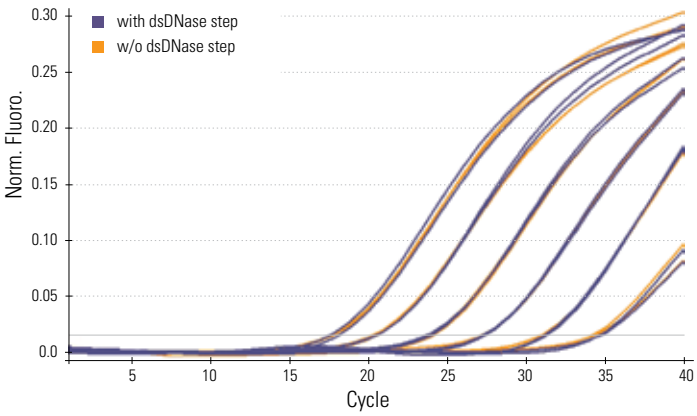
▲
Effective removal of genomic DNA using dsDNase.
Two-step RT-qPCR analysis of PBGD gene with (RT+) and without (RT-) reverse transcriptase. cDNA synthesis was performed from 0.2 ng total Jurkat RNA using Maxima First Strand cDNA Synthesis Kit with dsDNase (#K1671) or other kits including gDNA removal step. Flat RT- plot with Maxima kit indicates complete removal of contaminating gDNA, whereas RT- reaction with other suppliers' kits indicates amplification of residual gDNA.



Specificities of dsDNase

Substrate	Relative activity
dsDNA	100%
ssDNA	<0.03%
dsRNA	<0.01%
ssRNA	<0.01%

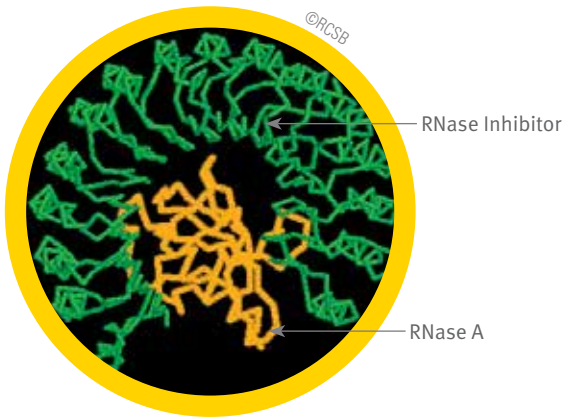
▲
Specificity towards double-stranded DNA.
dsDNase activity toward double- and single-stranded DNA and RNA oligonucleotides has been measured. Relative activity values indicate that dsDNase is highly specific to double-stranded DNA.



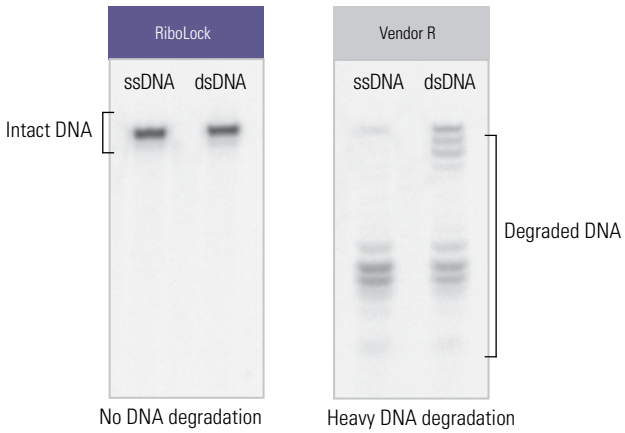
▲
dsDNase treatment does not compromise RNA quality and quantity.
Serial dilutions of total RNA were used in cDNA synthesis with Maxima First Strand cDNA Synthesis kits with and without dsDNase step. No changes in Cq values are observed between the compared protocols.

Efficient RNA protection: potent and pure RNase Inhibitor

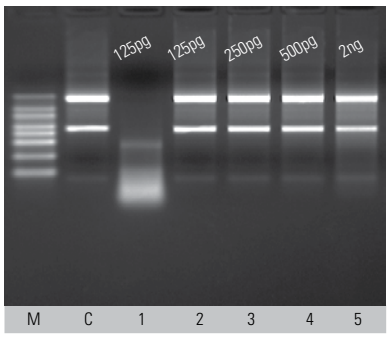
For RNA protection against RNases all Maxima First Strand cDNA Synthesis Kits include our engineered Thermo Scientific™ RiboLock™ RNase Inhibitor. It is a recombinant mammalian protein of non-human origin that completely protects RNA. The protein "locks" onto RNases and thus prevents RNA degradation. However, if the RNase Inhibitor is denatured; e.g., due to high temperatures, the RNases are released back to the reaction mix and can damage RNA. The engineered RiboLock RNase Inhibitor is active up to 55 °C. The high thermostability of RiboLock RNase Inhibitor is important for successful reverse transcription reactions at higher incubation temperatures.



▲
Thermo Scientifc RiboLock RNase Inhibitor "locks" onto RNase A to prevent RNA degradation.

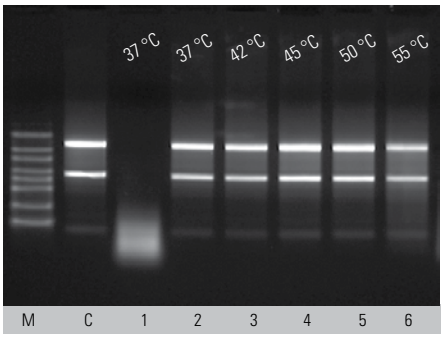


▲
Thermo Scientifc RiboLock RNase Inhibitor is free of contaminating DNase activities that may interfere with downstream applications. Single-stranded (ss) and double-stranded (ds) radiolabeled DNA fragments were incubated 4 hours at 37 °C with RiboLock RNase Inhibitor and a ribonuclease inhibitor from another vendor. The samples were analyzed by PAGE and phospho-imaging.



▲
Thermo Scientifc RiboLock RNase Inhibitor efficiently protects RNA and inhibits up to 2 ng/20 µL of RNase A. Aliquots (20 µL) of total human RNA (1 µg) were supplemented at 37 °C with 20 U of the RiboLock RNase Inhibitor and increasing amounts of RNase A.

M – Thermo Scientific™ RiboRuler™ High Range RNA Ladder, ready-to-use, (#SM1823)
C – total human RNA
1 – total human RNA with RNase A
2-5 – total human RNA with RiboLock RNase Inhibitor and RNase A



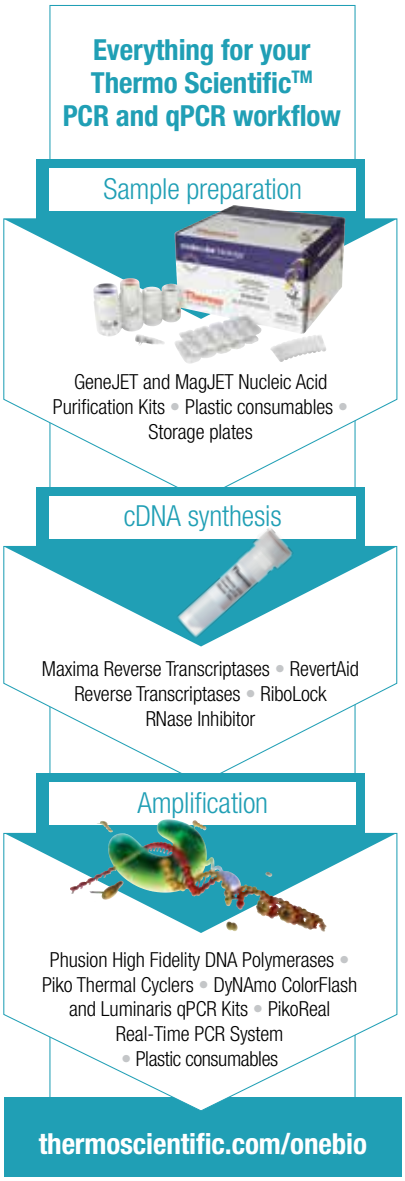
▲
Thermo Scientifc RiboLock RNase Inibitor exhibits high thermostability. Aliquots (20 µL) of total human RNA (1 µg) were supplemented with 20 U of the RiboLock RNase Inhibitor and 50 pg of RNase A and incubated at increasing temperatures.

M – RiboRuler High Range RNA Ladder, ready-to-use, (#SM1823)
C – total human RNA
1 – total human RNA with RNase A
2-6 – total human RNA with RiboLock RNase Inhibitor and RNase A

Ordering information

Product	Size	Order #
Maxima H Minus Reverse Transcriptase, 200 U/µL Supplied with 5× RT Buffer	2000 U	EP0751
	10 000 U	EP0752
	4 × 10 000 U	EP0753
Maxima H Minus First Strand cDNA Synthesis Kit Kit components: Maxima H Minus Enzyme Mix (Maxima H Minus RT and RiboLock RI), 5× RT Buffer, dNTP Mix, Oligo-(dT)18 Primer, Random Hexamer Primer and Water	20 rxns	K1651
	100 rxns	K1652
NEW **Maxima H Minus First Strand cDNA Synthesis Kit with dsDNase** Kit components: Maxima H Minus Enzyme Mix (Maxima H Minus RT and RiboLock RI), 5× RT Buffer, dNTP Mix, Oligo-(dT)18 Primer, Random Hexamer Primer, dsDNase, 10X dsDNase Buffer, and Water	20 rxns	K1681
	100 rxns	K1682
Maxima H Minus Double –Stranded cDNA Synthesis Kit Kit components: First Strand Enzyme Mix , 4× First Strand Reaction Mix, Second Strand Enzyme Mix , 5× Second Strand Reaction Mix , 0.5 M EDTA, RNase I, Control RNA, Oligo-(dT)18 Primer, Random Hexamer Primer, Water	10 rxns	K2561
Maxima Reverse Transcriptase, 200 U/µL Supplied with 5× RT Buffer	2000 U	EP0741
	10 000 U	EP0742
	4 × 10 000 U	EP0743
Maxima First Strand cDNA Synthesis Kit for RT-qPCR Kit components: Maxima Enzyme Mix (Maxima RT and RiboLock RI), 5× Reaction Mix (reaction buffer, dNTPs, Oligo-(dT)18 Primer and Random Hexamer Primer) and Water (nuclease-free)	50 rxns	K1641
	200 rxns	K1642
NEW **Maxima First Strand cDNA Synthesis Kit for RT-qPCR with dsDNase** Kit components: Maxima Enzyme Mix (Maxima RT and RiboLock RI), 5× Reaction Mix (reaction buffer, dNTPs, Oligo-(dT)18 Primer and Random Hexamer Primer), dsDNase, 10X dsDNase Buffer and Water	50 rxns	K1671
	200 rxns	K1672
RevertAid H Minus Reverse Transcriptase, 200 U/µL Supplied with 5× RT Buffer	10 000 U	EP0451
	5 × 10 000 U	EP0452
RevertAid H Minus First Strand cDNA Synthesis Kit Kit components: RevertAid H Minus RT, RiboLock RI, 5× RT Buffer, dNTP Mix, Oligo-(dT)18 Primer, Random Hexamer Primer, Control RNA, Control Primers and Water	20 rxns	K1631
	100 rxns	K1632
RevertAid Reverse Transcriptase, 200 U/µL Supplied with 5× RT Buffer	10 000 U	EP0441
	5 × 10 000 U	EP0442
RevertAid First Strand cDNA Synthesis Kit Kit components: RevertAid RT, RiboLock RI, 5× RT Buffer, 10 mM dNTP Mix, Oligo-(dT)18 Primer, Random Hexamer Primer, Control RNA, Control Primers and Water	20 rxns	K1621
	100 rxns	K1622
NEW **dsDNase** Supplied with 10x dsDNase Buffer	50 rxns	EN0771
RiboLock RNase Inhibitor, 40 U/µL	2500 U	EO0381
	4 x 2500 U	EO0382
	24 x 2500 U	EO0384

• Learn more at
thermoscientific.com/maxima



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