VWR®
We Enable Science

IT'S TIME FOR 5 PRIME

THE NEW DIMENSION IN PCR & PCR CLEAN-UP

5 PRIME

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PCR & RT-PCR

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PCR & RT-PCR

Choose The Best Enzyme for Your Application using the Selection Guide

Selection Chart PCR

PCR
- Standard
  - Classical reaction set-up
  - Convenient set-up
- Highest specificity & sensitivity
  - Classical reaction set-up
  - Convenient set-up

	Taq DNA Polymerase	5 PRIME MasterMix	HotMaster _Taq_ DNA Polymerase	5 PRIME HotMasterMix
Key feature	Self-adjusting Mg^{2+} buffer technology	Master mix format with _Taq_	Innovative Hot-Start/Cold-Stop Technology	MasterMix format with HotMaster _Taq_
Key benefit	Minimal PCR optimization	Convenient reaction setup for standard PCR	Highest PCR specificity and sensitivity	Convenient reaction setup for hot-start PCR
Additional features	Up to 8 kb fragments from genomic DNA	Minimize contamination No pipetting errors No miscalculation	Hot-Start without enzyme activation – optimal for fast PCR Up to 5 kb fragments from genomic DNA	Minimize contamination No pipetting errors No miscalculation
Applications	Standard PCR Standard RT-PCR	Routine standard PCR	Low-copy detection Multiplex PCR RT-PCR	Automated PCR reaction setup Routine hot-start PCR
Find more on page	7	8	9	11

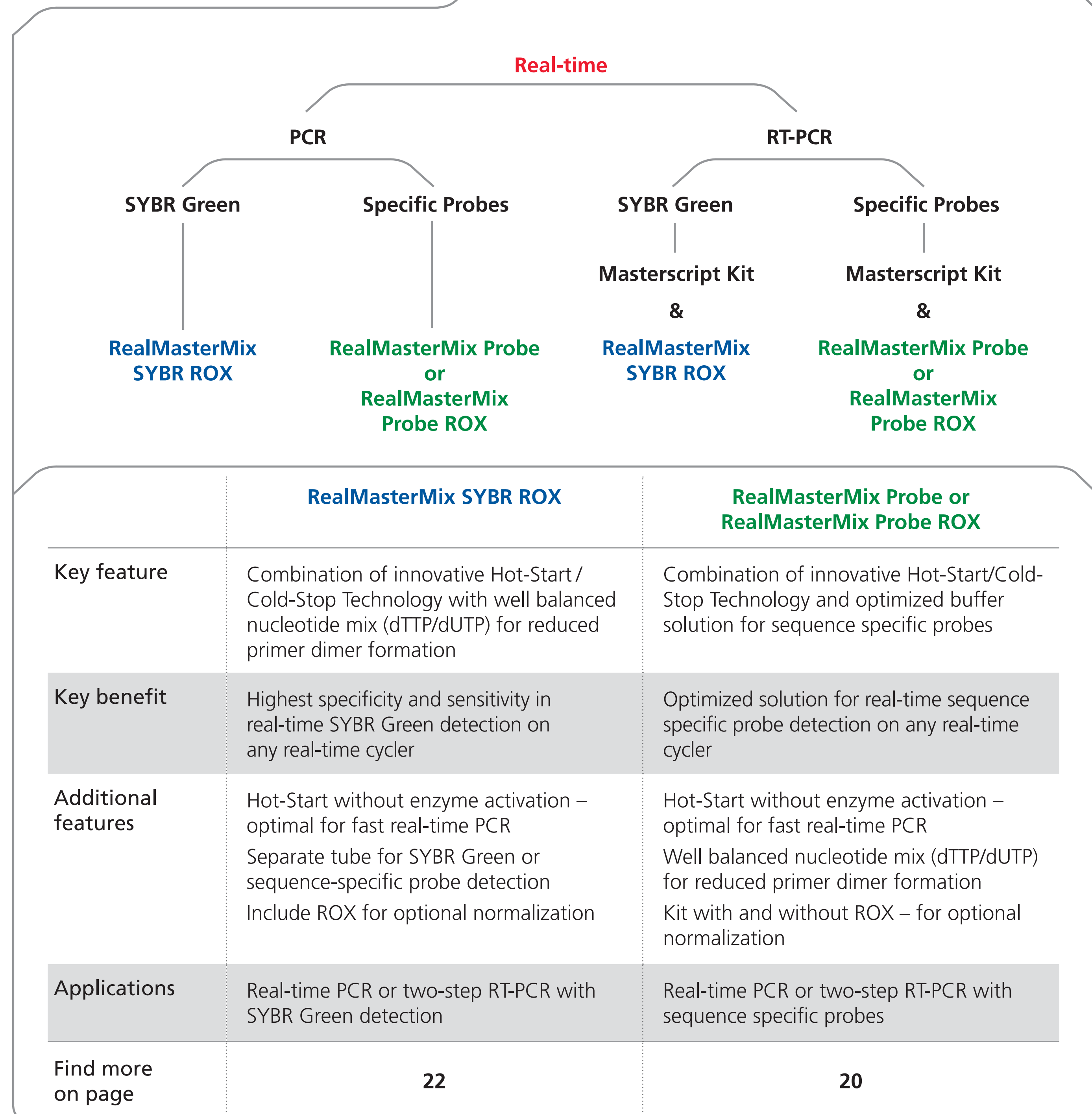
Selection Chart Special PCR Applications

	Master*Taq* Kit	Exact Polymerase	PCR Extender System
Key feature	Improved thermostability and processivity by *Taq*Master PCR Enhancer	Eliminated primer degradation through hot-start proofreading activity	Powerful proofreading-*Taq* mix combined with optimized buffer system
Key benefit	Robust PCR with higher tolerance against inhibitors	Robust proofreading amplification	Amplification of extremely long products up to 40 kb
Additional features	Higher yield	Very low error-rate Minimal PCR optimization	Reduced pH dependent target degradation Proofreading enzyme with low error rate
Applications	Contaminated samples e.g. soil Amplication of GC-rich templates and repetitive sequences	Error-free amplification up to 3 kb for cloning and mutagenesis	Amplification of long PCR fragments High-fidelity PCR for fragments >3 kb Amplification of GC-rich templates
Find more on page	12	14	13

Selection Chart Reverse Transcription & RT-PCR

	Masterscript Kit	Masterscript RT-PCR System
Key feature	High thermostable reverse transcriptase for cDNA synthesis at up to 65°C	Optimized all-in-one solution for all RT and RT-PCR applications
Key benefit	No secondary structures for full-length cDNA synthesis, up to 12 kb	Highly flexible: cDNA synthesis, two-step or one-step RT-PCR with one kit
Additional features	Eliminated intrinsic RNase H activity for reduced RNA template degradation – important for long cDNA synthesis Complete kit including Stop RNAse Inhibitor and dNTPs	Novel buffer system for optimal processivity of RT and PCR enzymes One-step RT-PCR up to 6 kb Two-step RT-PCR up to 12.5 kb Complete kit including Stop RNAse Inhibitor and dNTPs
Applications	Reverse Transcription from RNA with comlex secondary structures or high GC-content RT-PCR cDNA libraries	Sensitive two- and one-step RT-PCR Long-range two-step RT-PCR (12.5 kb)
Find more on page	16	18

Selection Chart Real-time PCR & RT-PCR



	RealMasterMix SYBR ROX	RealMasterMix Probe or RealMasterMix Probe ROX
Key feature	Combination of innovative Hot-Start / Cold-Stop Technology with well balanced nucleotide mix (dTTP/dUTP) for reduced primer dimer formation	Combination of innovative Hot-Start/Cold-Stop Technology and optimized buffer solution for sequence specific probes
Key benefit	Highest specificity and sensitivity in real-time SYBR Green detection on any real-time cycler	Optimized solution for real-time sequence specific probe detection on any real-time cycler
Additional features	Hot-Start without enzyme activation – optimal for fast real-time PCR Separate tube for SYBR Green or sequence-specific probe detection Include ROX for optional normalization	Hot-Start without enzyme activation – optimal for fast real-time PCR Well balanced nucleotide mix (dTTP/dUTP) for reduced primer dimer formation Kit with and without ROX – for optional normalization
Applications	Real-time PCR or two-step RT-PCR with SYBR Green detection	Real-time PCR or two-step RT-PCR with sequence specific probes
Find more on page	22	20

Minimal PCR optimization by self adjusting Mg^{2+} buffer technology

- Savings of time and effort – optimal PCR conditions provided
- High yields – robust amplification

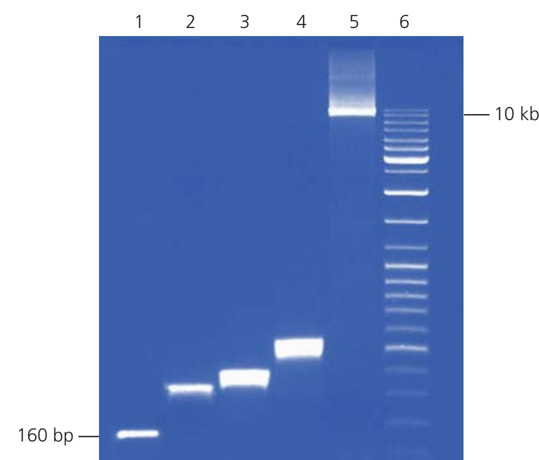
Savings of time and effort by optimized PCR conditions

The 5 PRIME *Taq* DNA Polymerase is provided with a 10x self-adjusting Mg^{2+} buffer. The formulation adjusts the Mg^{2+} concentration automatically, eliminating the need to optimize this critical component. The self-adjusting buffer acts by weakly chelating Mg^{2+} ions: excess Mg^{2+} is bound by the chelating agent and, as free Mg^{2+} decreases, is released and available to the *Taq* polymerase. The self-adjusted Mg^{2+} concentration is pre-optimized for any template–primer system, saving time and precious samples.

High yields through robust amplification

5 PRIME *Taq* DNA Polymerase is an ultra-pure, thermostable recombinant DNA Polymerase. Its recombinant nature ensures the utmost purity, reproducibility, and processivity. The enzyme's thermostability meets the requirements of even the most specialized PCR applications. An initial incubation time of 20 min. at 95°C before starting the PCR reaction does not significantly influence the enzymes activity. Together with the self adjusting Mg^{2+} buffer technology the robust amplification provides the highest yields.

5 PRIME *Taq* and the self adjusting Mg^{2+} buffer are also included in the 5 PRIME MasterMix (see page 8).



1 160 bp fragment of apolipoprotein B (genomic DNA)
2 360 bp fragment of apolipoprotein B (genomic DNA)
3 400 bp fragment of *Taq* Polymerase gene (60% GC, plasmid DNA)
4 547 bp fragment, GC-rich (70% GC, genomic DNA)
5 10 kb fragment from the genome of lambda phage
6 DNA Marker

Fig. 1 I Amplification of fragments with different lengths using *Taq* DNA Polymerase.

Kit content

Component/Units	*Taq* DNA Polymerase 100 U	250 U	1000 U	5000 U = 5 x 1000 U
Taq DNA Polymerase (5 U/µl)	20 µl	50 µl	4 x 50 µl	20 x 50 µl
10x *Taq* Buffer advanced	1 x 1.8 ml	1 x 1.8 ml	3 x 1.8 ml	15 x 1.8 ml
25 mM Magnesium Solution	1 x 1.5 ml	1 x 1.5 ml	2 x 1.5 ml	10 x 1.5 ml
Ref. No.	**2200000**	**2200010**	**2200020**	**2200030**

Robust PCR amplification with minimal reaction setup

- Minimize handling – ready-to-use mastermix format
- Convenience – storage at 4°C eliminates freeze-thaw cycles

Minimal handling with ready-to-use master mix format

5 PRIME MasterMix (2.5x) is a ready-to-use PCR mix that offers high reproducibility when processing large numbers of samples. Only primers and template need to be added to the 2.5x concentrate, decreasing the number of time-consuming pipetting steps. This format not only reduces the likelihood of errors and the risk of contamination, but it also increases precision and sample throughput.

The 5 PRIME MasterMix also contains the self-adjusting Mg^{2+} buffer technology (described on page 7).

This formulation adjusts the Mg^{2+} concentration automatically, eliminating the need for optimizing this critical component.

Convenient 4°C storage eliminates freeze-thaw cycles

The MasterMix does not need to be stored frozen, eliminating the time-consuming thawing process and the resulting reduction in performance. The MasterMix is stable at 4°C for at least 3 month when stored properly.

The 5 PRIME MasterMix is a 2.5x concentrated mix with final concentrations in a 50 µl PCR reaction of 1.25 U/50 µl *Taq* DNA Polymerase, 45 mM KCl, 2.5 mM Mg^{2+}, and 200 µM of each dNTP.

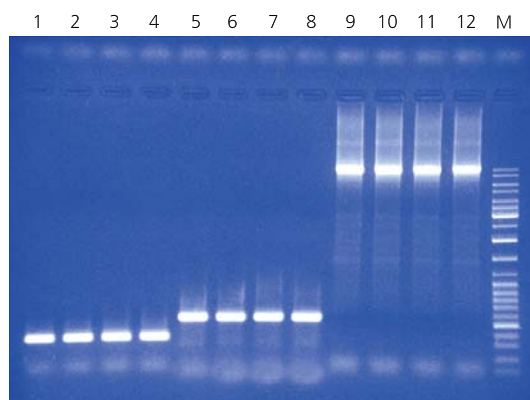


Fig. 2 I Stability test, after storage of 5 PRIME Master Mix for 48 hours at -20°C, 2–8°C, 20–27°C and 37°C, three different fragements were amplified. 1-4 360 bp fragment of apolipoprotein B (genomic DNA), 5–8 547 bp fragment, GC-rich (70% GC, genomic DNA). 9–12 10 kb fragment of genome of lamda phage, **M** DNA marker

Kit content

Component/Reactions & Units	5 PRIME MasterMix 100 Rxns* = 125 U	5 PRIME MasterMix 1,000 Rxns* = 1,250 U
2.5x MasterMix Solution	2 x 1 ml	20 x 1 ml
25 mM Magnesium Solution	1 x 1.5 ml	4 x 1.5 ml
Ref. No.	**2200100**	**2200110**

* 1 reaction = 50 µl

Innovative Hot-Start/Cold-Stop technology for highly specific hot-start PCR without enzyme activation

- Optimal results – highly specific amplification
- Savings of time and effort – optimal PCR conditions provided
- Excellent for fast PCR protocols – no enzyme activation

Optimal results with highly specific amplification

Hot-start is a well established method for improving PCR specificity. HotMaster *Taq* DNA polymerase is designed to reduce or eliminate any non-specific products that result from mispriming during PCR.

Conventional hot-start technologies, such as antibody-mediated inhibition or chemical blocking of DNA polymerases, have limitations, such as long initial activation steps that can reduce the performance of the DNA polymerase and compromise specificity control.

To avoid this disadvantages, HotMaster *Taq* DNA Polymerase features a superior hot-start PCR technology: a temperature-dependent inhibitory ligand completely inhibits *Taq* polymerase activity at low temperatures. At high temperatures, the inhibitor is released and full *Taq* activity is immediately restored. Since the process is reversible, the "cold stop" aspect of the inhibitor has the potential to block enzyme activity in every low-temperature cycle of the PCR, ensuring optimal results.
Unlike standard *Taq* polymerases, HotMaster *Taq* reactions can be set up at room temperature.

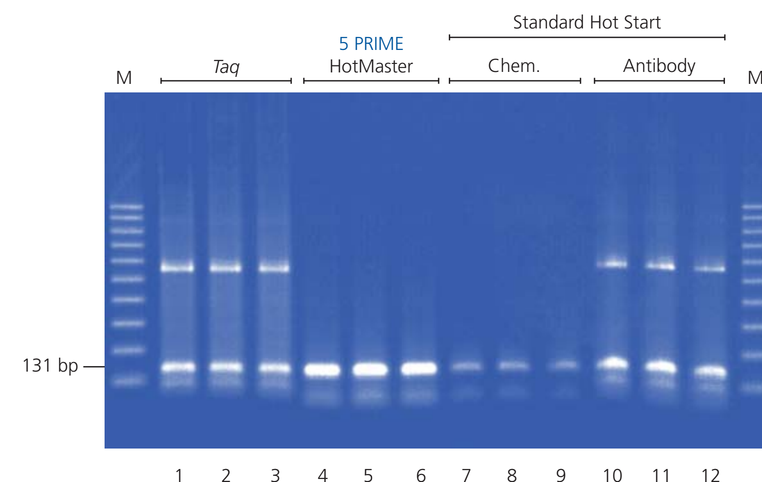


Fig. 3 I Fast PCR Amplification of a 131 bp fragment of the human TFN gene with standard *Taq*, HotMaster *Taq* and conventional Hot Start enzymes. Used protocol: 1 sec 95°C denaturation, 1 sec 55°C annealing, 5 sec 72°C extension. Initial dentauration was 2 min at 95°C prior to PCR or 10 min for the chemically modified enzyme respectively.

Savings of time and effort by optimized PCR conditions

The HotMaster *Taq* DNA Polymerase is provided with a 10x self-adjusting Mg^{2+} buffer (described on page 7). The formulation adjusts the Mg^{2+} concentration automatically, eliminating the need for optimizing this critical component.

Excellent for fast PCR protocols – no enzyme activation

The initial heat-activation step required by standard hot-start PCR enzymes prior to cycling, typically up to 15 minute incubation at 95°C, can reduce enzyme activity and result in lower yield of PCR product.

HotMaster *Taq* does not require an initial heat-activation step and can efficiently amplify short fragments as well as fragments up to several kb (e.g. up to 5 kb from genomic DNA) with high specificity. This combination of high efficiency and high specificity guarantees detection of extremely low levels of target DNA. Even the presence of high levels of non-template DNA, HotMaster *Taq* can amplify less than 10 target DNA molecules.

Kit content

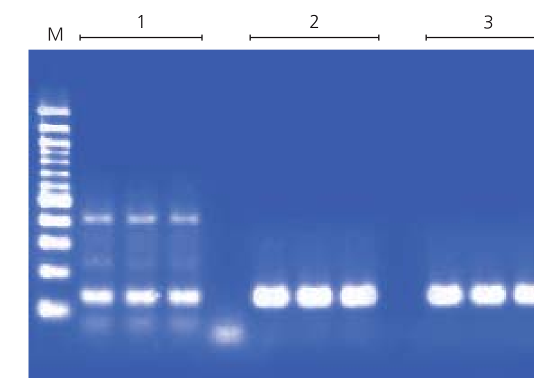
Component/Units	HotMaster™ *Taq* DNA Polymerase 100 U	250 U	1000 U	5000 U = 5 x 1000 U
HotMaster™ *Taq* DNA Polymerase (5 U/µl)	20 µl	50 µl	4 x 50 µl	20 x 50 µl
10x HotMaster™ *Taq* Buffer with 25 mM Mg^{2+}	1 x 1.8 ml	1 x 1.8 ml	3 x 1.8 ml	15 x 1.8 ml
Ref. No.	**2200300**	**2200310**	**2200320**	**2200330**

Highly specific hot-start PCR with minimal reaction setup

- Optimal results – highly specific amplification
- Minimal handling – ready-to-use mastermix format
- Convenience – storage at 4°C eliminates freeze-thaw cylces

Optimal results with highly specific amplification

5 PRIME HotMasterMix is a ready-to-use reagent mix for performing hot-start PCR in a highly convenient format. HotMaster *Taq* DNA polymerase, an integral component of the master mix, is designed to reduce or eliminate any non-specific products that may result from mispriming during PCR (described on page 9).



M 100 bp Marker
1 Standard *Taq*
2 HotMaster *Taq*
3 HotMasterMix

Fig. 4 | Amplification of a 131 bp TNF fragment.

Minimal handling with ready-to-use master mix format

5 PRIME HotMasterMix (2.5x) is a ready-to-use PCR mix that offers high reproducibility when processing large numbers of samples. Only primer and template need to be added to the 2.5x concentrate, decreasing the number of time-consuming pipetting steps. This format not only reduces the likelihood of errors and the risk of contamination, but it also increases precision and sample throughput. The HotMasterMix also contains the self-adjusting Mg^{2+} buffer technology (described on page 7). This formulation adjusts the Mg^{2+} concentration automatically, eliminating the need for optimizing this critical component.

Convenient storage at 4°C eliminates freeze-thaw cylces

The MasterMix does not need to be stored frozen, eliminating the time-consuming thawing process and the resulting reduction in performance.

The 5 PRIME HotMasterMix is a 2.5X concentrated with final concentrations in a 50 µl PCR reaction of 1.0 U/50 µl *Taq* DNA Polymerase, 45 mM KCl, 2.5 mM Mg^{2+}, and 200 µM of each dNTP.

Kit content

Component/Reactions & Units	5 PRIME HotMasterMix 100 Rxns* = 100 U	1,000 Rxns* = 1000 U
2.5x HotMasterMix Solution	2 x 1 ml	20 x 1 ml
25 mM Magnesium Solution	1 x 1.5 ml	4 x 1.5 ml
Ref. No.	**2200400**	**2200410**

* 1 reaction = 50 µl

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Available from: VWR INTERNATIONAL, PTY LTD.
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Master™ *Taq* DNA Polymerase

Contains *Taq*Master PCR Enhancer that provides robust amplification of contaminated and difficult samples

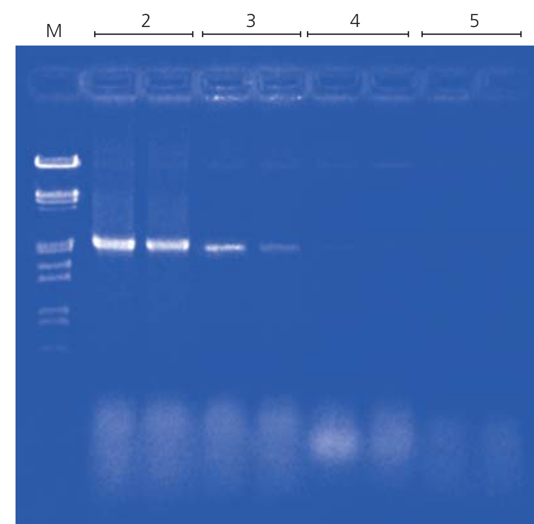
- ⇨ Amplification of difficult samples – improved *Taq* processivity with *Taq*Master PCR Enhancer
- ⇨ Robust PCR – high tolerance of PCR inhibitors

Amplification of difficult samples with the improved processivity provided by the *Taq*Master PCR Enhancer

The Master*Taq* Kit ensures high-yield PCR when using difficult template DNA. The *Taq*Master PCR Enhancer (5x concentrated) is a buffer additive that improves thermostability (enzyme half-life) and processivity of *Taq* polymerase by stabilizing the enzyme during PCR. In contrast to other PCR-enhancing additives, *Taq*Master PCR Enhancer is chemically stable and inert. It's uses does not affect primer annealing or template denaturation, allowing easy adaptation of existing protocols.

Robust PCR with high tolerance to PCR inhibitors

5 PRIME Master*Taq* Kits are designed to ensure improved yield and reliable product amplification. The presence of *Taq*Master PCR Enhancer protects the enzyme against exogenous activity-inhibiting contamination (e.g., humic acid from soil samples, blood compounds, etc.). Increased yields and reproducible results are obtained when amplifying impure samples or GC-rich templates with *Taq*Master PCR Enhancer. *Taq*Master PCR Enhancer is available separately and as a kit component.



1 Marker: λ Eco RI *Hind* III
2 5 PRIME Master*Taq*
3 5 PRIME *Taq*
4 *Taq*, Supplier A
5 *Taq*, Supplier B

Fig. 5 | Amplification of SSU rRNA gene from genomic DNA.

Kit content

Component/Units	100 U	250 U	500 U	1000 U
	Master*Taq* Kit			
Taq DNA Polymerase (5 U/µl)	20 µl	50 µl	2 x 50 µl	4 x 50 µl
10x *Taq* Buffer with Mg^{2+}	1 x 1.8 ml	1 x 1.8 ml	2 x 1.8 ml	3 x 1.8 ml
25 mM Magnesium Solution	1 x 1.5 ml	1 x 1.5 ml	1 x 1.5 ml	2 x 1.5 ml
5x *Taq*Master PCR Enhancer	1 x 1.7 ml	2 x 1.7 ml	3 x 1.7 ml	6 x 1.7 ml
Ref. No.	**2200200**	**2200210**	**2200220**	**2200230**

PCR Extender System

Amplification of extremely long products up to 40 kb with high yields

- ⇨ High yields from complex targets – efficient amplification up to 40 kb
- ⇨ Optimized processivity – two buffer systems for long and high-fidelity PCR

High yields from complex targets with efficient amplification up to 40 kb

The PCR Extender System combines a powerful polymerase blend with an innovative two buffer system for efficient amplification of products ranging from 100 bp up to 40 kb, including GC-rich targets and other difficult templates. The PCR Extender Polymerase Mix is a blend of thermostable DNA polymerases that includes a processivity-enhancing factor. This unique mixture enables extremely high extension rates, maximum proofreading fidelity and high amplification efficiency, even when using complex templates. This features ensure the optimal amplification of PCR products for cloning and mutagenesis.

Optimized processivity with a two buffer system for high-fidelity PCR of long targets

The two buffer systems provided in the PCR Extender System, the Tuning Buffer and the PCR Extender Buffer, provide the unique zwitterionic formulation that improves pH-maintenance at high temperatures (72–94°C). This system reduces pH-driven template degradation to a minimum. The Tuning Buffer is designed for high-fidelity PCR applications and robust amplification of genomic targets >20 kb and episomal targets up to 40 kb without organic co-solvents. The PCR Extender Buffer is designed for high-fidelity amplifications of smaller targets ranging from 100 bp to 10 kb genomic DNA and up to 15 kb plasmid or phagemid.

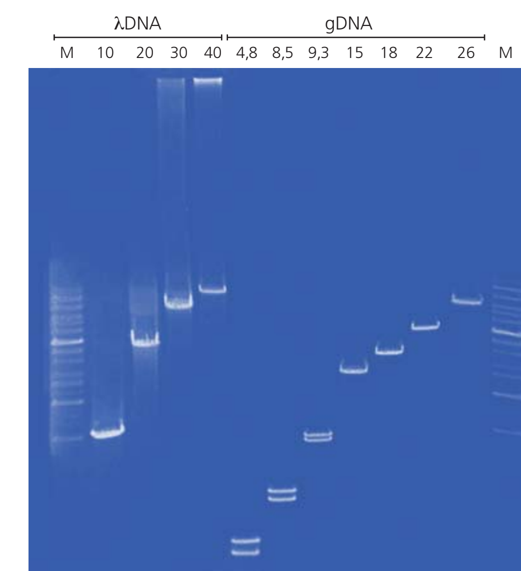


Fig. 6 | Amplification of different fragments (4.8-40 kb) from lambda and human genomic DNA using the PCR Extender System. Double bands correspond to different allele variants.

Kit content

Component/Units & Reactions	100 U (50 - 100 Rxns)	200 U (100 – 200 Rxns)	500 U (250 – 500 Rxns)
	PCR Extender System		
PCR Extender Polymerase Mix (5U/µl)	20 µl	2 x 20 µl	5 x 20 µl
10x Tuning Buffer with Mg^{2+}	1 x 1.5 ml	1 x 1.5 ml	1 x 1.5 ml
10x PCR Extender Buffer with Mg^{2+}	1 x 1.5 ml	1 x 1.5 ml	2 x 1.5 ml
25 mM Magnesium Solution	1 x 1.5 ml	1 x 1.5 ml	1 x 1.5 ml
Ref. No.	**2200500**	**2200510**	**2200520**

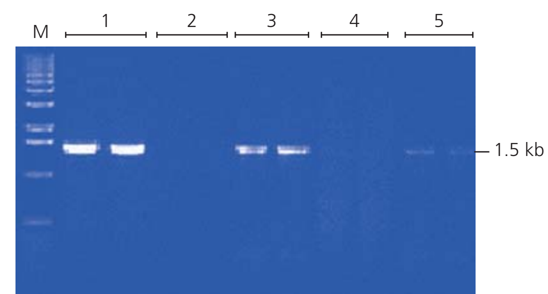
For high-fidelity hot-start PCR

- Robust performance – modified enzyme to prevent primer degradation
- High fidelity – accurate products by efficient proofreading activity
- Minimal optimization – Self adjusting Mg^{2+} buffer and 5 P-Solution

Robust performance eliminating primer degradation

Proofreading enzymes exhibit a 3'– 5' exonuclease activity that removes incorrectly incorporated bases. These enzymes can also degrade primers during reaction setup and the start of PCR, causing nonspecific priming, smearing, or amplification failure.

Exact Polymerase is chemically modified to exhibit no polymerase or exonuclease activity before activation. After a simple hot-start, both enzyme properties are activated and PCR proceeds with high proofreading amplification. Due to the unique modification, primers remain intact and amplification from mispriming cannot occur during setup. As a result, Exact Polymerase is more robust than typical proofreading enzymes, ensuringe reliable and specific PCR with minimal PCR optimization.



1 Exact Polymerase
2 Supplier L
3 Supplier R (Taq/proofreading mix)
4 Supplier R
5 Supplier S

Fig. 7 I Amplification of 1.5 kb human hypocanthine ribosyl transferese fragment from genomic DNA with proofreading polymerases or *Taq*/proofreading mixtures from differnet suppliers. **M** DNA marker.

Accurate amplification guaranteed by efficient proofreading activity

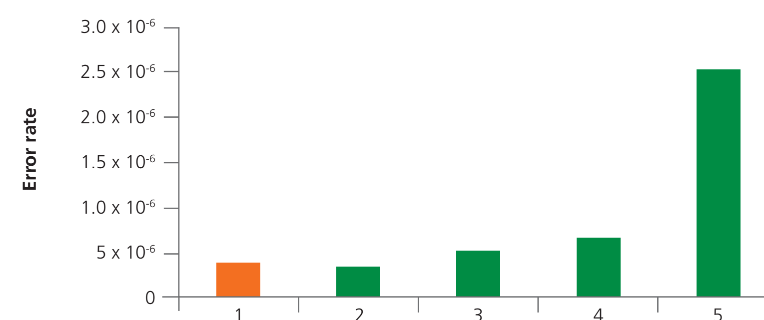
The proofreading activity of Exact Polymerase ensures that products are amplified with an extremely low error rate: Several error-rate measurements indicate that Exact Polymerase is more than 10 times more accurate than *Taq* DNA Polymerase.
The robust performance gives trouble-free amplification with minimum errors.

Exact Polymerase specifications

Concentration:	2.5 U/µl
5' – 3' exonuclease activity:	No
3' – 5' exonuclease activity:	Yes
Extra A addition (terminal transferase activity):	Minimal
Half-life:	> 4 hours at 95°C
Substrate analogs:	Use of dUTP is not recommended.
Fidelity:	2.34 – 3.6 x 10^{-6} misincorporated bases per PCR product doubling

Minimal optimization with self-adjusting Mg^{2+} buffer and 5P-Solution

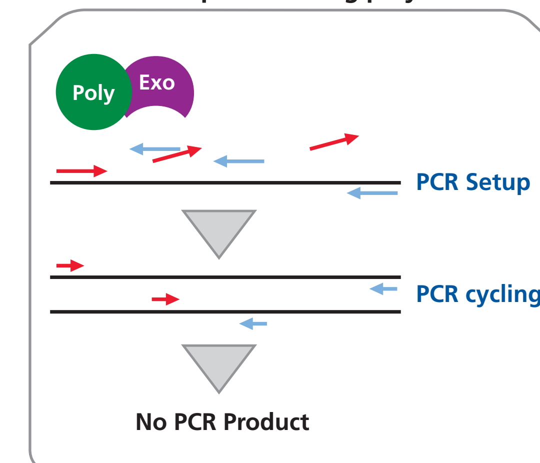
Exact Polymerase is provided with a 10x self-adjusting Mg^{2+} buffer (described on page 7). This formulation adjusts the Mg^{2+} concentration automatically, eliminating the need for optimizing this critical component. For templates with a high degree of secondary structure or a high GC content, Exact Polymerase is provided with 5P-Solution. This innovative PCR reagent modifies the melting behavior of DNA to improve amplification of problematic templates.



1 Exact Polymerase
2 Supplier S
3 Supplier N
4 Supplier R (*Taq*/proofreading polymerase mix)
5 Supplier A *Taq*

Fig. 8 I Error rates were determined using a beta-galactosidase PCR mutation assay.

A Other proofreading polymerases



B Exact Polymerase

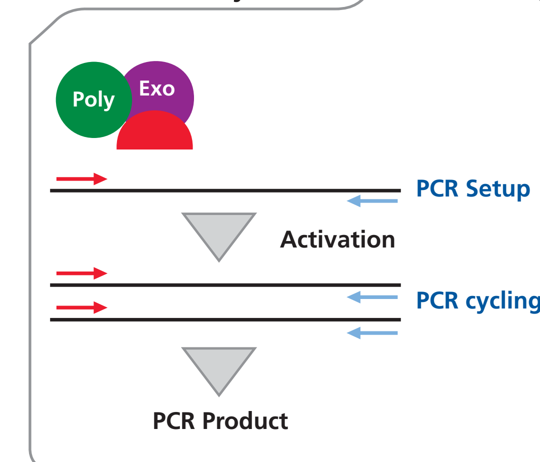


Fig. 9 I Graphic of primer degradation by proofreading polymerases with exonuclease activity (Exo) and a polymerase activity (Poly).

Kit content

Component/Units & Reactions	Exact Polymerase 100 U = 40 Rxns*	Exact Polymerase 500 U = 200 Rxns*
Exact Polymerase	40 µl	200 µl
10x Exact PCR Buffer	1 x 1.0 ml	1 x 1.0 ml
25 mM Mg_2SO_4	1 x 1.0 ml	1 x 1.0 ml
5x 5P Solution	400 µl	1 x 2.0 ml
Ref. No.	**2200600**	**2200610**

* 1 reaction = 50 µl

Extremely thermostable reverse transcriptase for efficient full-length cDNA synthesis

- ⇨ Full length cDNA synthesis up to 12 kb – through highly thermostable reverse transcriptase
- ⇨ Detection of low RNA amounts – highly sensitive reverse transcription
- ⇨ Only primer addition – complete kit with Stop RNase Inhibitor and dNTPs

Full-length cDNA synthesis up to 12 kb with a highly thermostable reverse transcriptase

Reverse transcription (RT) and RT-PCR are the most popular techniques for RNA analysis. The Masterscript Kit enables the full range of RNA-related applications. The Masterscript RT Enzyme features a recombinant, highly processive, homo-dimeric, viral reverse transcriptase for synthesizing cDNAs of up to 12.5 kb. The enzyme can be used with low-abundance template RNA. The post-translationally modified Masterscript RT is thermostable at temperatures up to 65°C, allowing cDNA synthesis from RNA templates with a complex secondary structure or high GC content. The unique buffer provided in the kit is fully compatible with downstream PCR amplification, allowing easy two-step RT-PCR and real-time RT-PCR. The unique buffer provided in the kit is fully compartible with different primer types, e.g. gene-specific, oligo dT- or random primers and not interferes with any downstream PCR amplification.

Highly sensitive reverse transcription by detection of low-abundance RNA

The Masterscript RT is post-translationally modified to reduce intrinsic temperature-dependent RNase H activity to a level that has no impact on full-length cDNA synthesis. This modification ensures highly sensitive reverse transcription, even when using very low starting amounts of RNA.

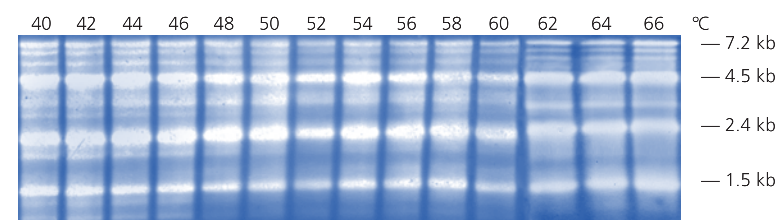


Fig. 10 I Reverse transcription of an RNA ladder at various temperatures using Masterscript Kit.

Complete kit with Stop RNase Inhibitor and dNTPs

The Masterscript Kit contains all necessary components to perform optimal first-strand cDNA synthesis. To efficiently prevent RNA degradation, the kit includes the Stop RNase Inhibitor, an extremely potent inhibitor of ribonucleases. The protein is of non-human origin and prevents RNA degradation by inhibition of >90% RNAase activity (described on page 24).

Kit content

	Masterscript Kit	
Component/Units & Reactions	**750 U = 50 Rxns***	**3000 U = 200 Rxns***
Masterscript RT Enzyme (15 U/µl)	50 µl	4 x 50 µl
RT-PCR Buffer with 25 mM Magnesium	1 x 1.0 ml	1 x 1.0 ml
Stop RNase Inhibitor Solution	50 µl	4 x 50 µl
10 mM dNTP Mix	100 µl	4 x 100 µl
RNase-free H_2O	1 x 1.8 ml	1 x 1.8 ml
Ref. No.	**2201000**	**2201010**

* 1 reaction = 50 µl

Optimized all-in-one solution for cDNA synthesis, one-step and two-step RT-PCR

- ⇨ Maximum flexibility – high yield reverse transcription and optimized PCR reaction
- ⇨ Long-range RT-PCR up to 12 kb – optimized proofreading PCR enzyme mix
- ⇨ Only primer addition – complete kit with Stop RNase Inhibitor and dNTPs

Maximized flexibility with high-yield reverse transcription and optimized PCR

The Masterscript RT-PCR System is designed to provide maximum flexibility with just one kit: first strand cDNA synthesis and either one- or two-step RT-PCR. The innovative product design and buffer provide easy handling.

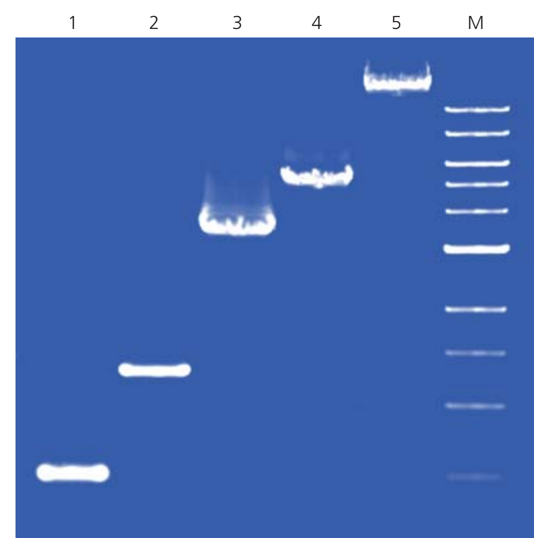
The novel Masterscript RT-PCR buffer system allows optimal processivity of both the reverse transcriptase and DNA polymerase. With interchangeable buffers, you can perform cDNA synthesis in one- or two-step RT-PCR, the choice is yours.

Long RT-PCR up to 12 kb with proofreading PCR enzyme mix

The PCR enzyme mix in the Masterscript RT-PCR System is a blend of DNA polymerases with a high extension rate and a DNA polymerase with proofreading function. In combination with the Tuning Buffer, the Masterscript RT-PCR Enzyme Mix guarantees robust and high-fidelity amplification of very long cDNAs. Using a one-step protocol, cDNAs of up to 6 kb can extended with great sensitivity. The optional two-step RT-PCR protocol is available to amplify extremely long cDNAs of up to 12.5 kb or difficult or low-abundant templates. In addition, the Masterscript RT-PCR buffer is formulated to adjust the Mg^{2+} concentration automatically, so that there is never a need for optimization of this critical component.

Complete kit with Stop RNase Inhibitor and dNTPs

The Masterscript RT-PCR Kit contains all necessary components to perform an optimal first-strand cDNA synthesis, one- or two-step RT-PCR reactions except primers. To efficiently prevent RNA degradation the kit includes the Stop RNase Inhibitor, an extremely potent inhibitor of ribonucleases. The protein is of non-human origin and prevents RNA degradation by inhibition of >90% RNAase activity (described on page 24).



1 Tubulin (Mouse kidney)
2 TFRC (HeLa-S3)
3 POLE (HeLa-S3)
4 TSC1 (HeLa-S3)
5 Dynein (Mouse kidney)
M DNA Marker
TFRC and dynein are rare transcripts

Fig. 11 | Two-step RT-PCR of various mRNA templates from different sources (cell lines from mouse and human).

Kit content

	Masterscript RT-PCR System	
Component/Reactions	**20 Rxns**	**100 Rxns**
Masterscript RT Enzyme (15 U/µl)	10 µl (150 U)	50 µl (750 U)
Masterscript PCR Enzyme Mix (5 U/µl)	10 µl (50 U)	50 µl (250 U)
RT-PCR Buffer with 25 mM Mg^{2+}	1 x 1.0 ml	1 x 1.0 ml
Stop RNase Inhibitor Solution	20 µl	50 µl
10x Tuning Buffer with 25 mM Mg^{2+}	1 x 1.5 ml	1 x 1.5 ml
10 mM dNTP Mix	50 µl	5 x 50 µl
RNase-free H_2O	1 x 1.8 ml	1 x 1.8 ml
Ref. No.	**2201100**	**2201110**

For highly sensitive and specific probe-based, real-time PCR detection

⇨ Superior sensitivity and specificity – by innovative Hot-Start/Cold-Stop technology
⇨ Ideal for fast real-time PCR – no enzyme activation step required
⇨ Accurate results – anti primer-dimer formation with optimized nucleotide formulation
⇨ Easy set-up – Ready-to-use master mix with or without ROX

Superior sensitivity and specificity with innovative Hot-Start/Cold-Stop technology

RealMasterMix Probe is the ideal master mix for sequence-specific, probe-based real-time PCR assays. The RealMasterMix Probe contains the HotMaster *Taq* DNA Polymerase which features superior hot-start PCR technology (described on page 9).

Ideal for fast real-time PCR as no enzyme activation step is required

HotMaster *Taq* DNA Polymerase, contained in the RealMasterMix Probe, does not require heat activation prior to cycling. Other hot-start PCR enzymes require activation with 15 minute incubation at 95 °C, which affects enzyme activity and can result in lower PCR product yields. By eliminating the need for initial heat activation, the HotMaster *Taq* and the RealMasterMix Probe are the reagents of choice for short PCR run times.

Reduced primer–dimer formation with optimized nucleotide formulation for accurate results

The patent-pending anti-primer–dimer feature significantly reduces nonspecific primer annealing. A well balanced nucleotide formulation, with an optimized ratio of dTTP and dUTP, increases the specificity and sensitivity of the reaction. The formulation also allows the performance of Uracil-N-Glycosylase (UNG) treatment at ≤ 40°C. In addition, the RealMasterMix Probe buffer contains the self-adjusting Mg^{2+} buffer technology which eliminates the need for real-time PCR optimization (described on page 7).

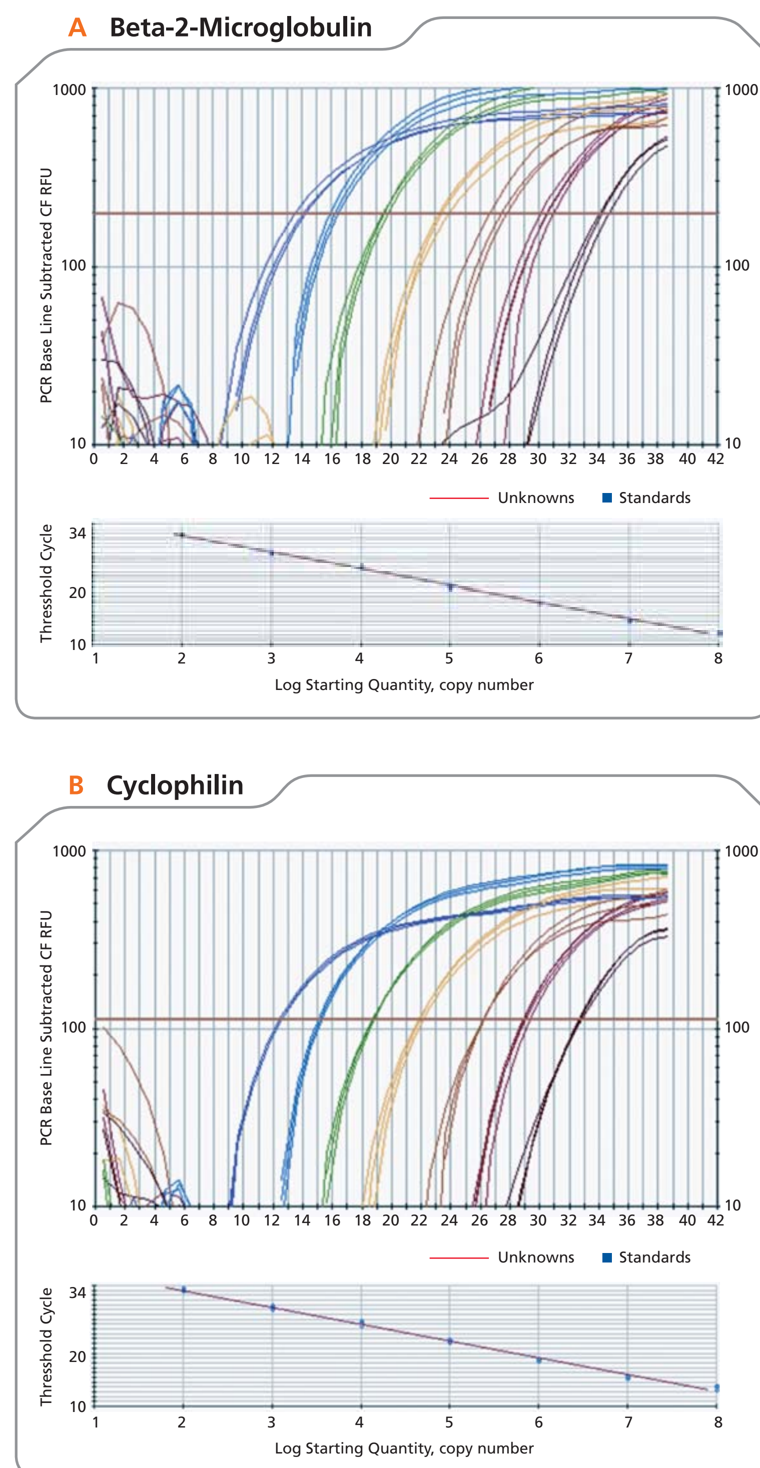


Fig. 12 I RealMasterMix Probe amplification of a dual-plex reaction for Beta-2-Microglobulin (B2M) and Cyclophilin in three replicates with high degree of efficiency (> 93%)

Easy set-up with a ready-to-use master mix with or without ROX

For easy and fast handling, all components of the RealMasterMix Probe, except for template and primers/probes, are contained in one tube. The master mix can be used with any real-time cycler and is available in formulations with or without ROX, a non-DNA-binding passive-reference dye used for normalization.

Additional magnesium solution is provided to optimize multiplex PCR with more than two targets. The use of HotMaster *Taq* in the master mix allows reactions to be set up easily at room-temperature without any risk of non specific amplification. The RealMasterMix Probe is stable at 4°C for at least 3 months when stored properly. Cycles of freezing and thawing the solution are eliminated, saving time.

Kit content

	RealMasterMix Probe 2.5x	
Components/Reactions	**200 Rxns***	**2000 Rxns* = 10 x 200 Rxns**
2.5x RealMasterMix Probe (0.1 U/µl HotMaster *Taq* DNA Polymerase, 12.5 mM Magnesium Acetate (5.0 mM final), 1.0 mM dNTPs with dUTP (0.4 mM final) in the 2.5x MasterMix)	4 x 1.0 ml	40 x 1.0 ml
25 mM Magnesium Solution	1 x 1.5 ml	10 x 1.5 ml
Ref. No.	**2200700**	**2200710**

	RealMasterMix Probe ROX 2.5x	
Components/Reactions	**200 Rxns***	**2000 Rxns* = 10 x 200 Rxns**
2.5x RealMasterMix Probe ROX (0.1 U / µl HotMaster *Taq* DNA Polymerase,12.5 mM Magnesium Acetate (5.0 mM final), 1.0 mM dNTPs with dUTP (0.4 mM final) in the 2.5x MasterMix)	4 x 1.0 ml	40 x 1.0 ml
25 mM Magnesium Solution	1 x 1.5 ml	10 x 1.5 ml
Ref. No.	**2200720**	**2200730**

* 1 reaction = 50 µl

Accurate real-time quantification using SYBR Green, and also sequence-specific probe detection

- ⇨ Superior sensitivity and specificity – by innovative Hot-Start/Cold-Stop technology
- ⇨ Accurate results – anti primer-dimer formation with optimized nucleotide formulation
- ⇨ Ideal for fast real-time PCR – no enzyme activation step required
- ⇨ Easy set-up with a flexible system – Ready-to-use master mix with ROX for SYBR Green or sequence-specific probe detection

Superior sensitivity and specificity with innovative Hot-Start/Cold-Stop technology

RealMasterMix SYBR ROX is the ideal master mix for SYBR Green-based real-time PCR assays. SYBR Green dye binds to double-stranded DNA, including any non specific amplification products and primer–dimers formed during real-time PCR. Binding to any molecules other than the amplification product will decrease the accuracy of target quantification. The RealMaster Mix SYBR ROX, containing HotMaster *Taq* DNA Polymerase (described on page 9), provides a superior Hot-Start/Cold-Stop technology, which prevents non specific amplification during PCR setup and in every PCR cycle. The use of this enzyme guarantees the highest specificity and sensitivity.

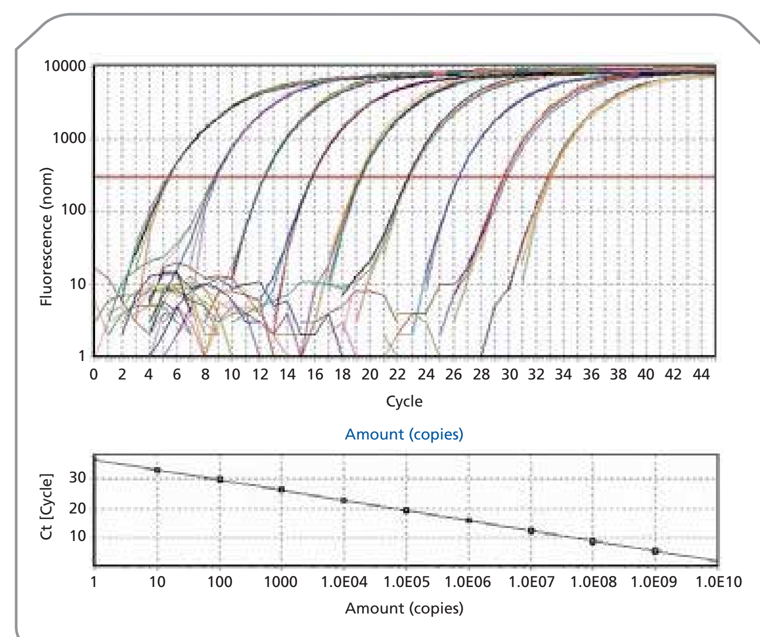


Fig. 13 | 10-fold serial dilution of lambda DNA in a SYBR Green I assay using RealMasterMix SYBR ROX 2.5x in a 37 min runtime protocol.

Reducing primer–dimer formation with optimized nucleotide formulation for accurate results

The patent-pending anti-primer–dimer feature significantly reduces nonspecific primer annealing (described on page 20). In addition, the RealMasterMix SYBR ROX buffer contains the self-adjusting Mg^{2+} buffer technology which eliminates the need for real-time PCR optimization (described on page 7).

Ideal for fast real-time PCR without enzyme activation step

HotMaster *Taq* DNA Polymerase in the RealMasterMix SYBR ROX does not require heat activation prior to cycling, making it the best choice for short PCR run times.

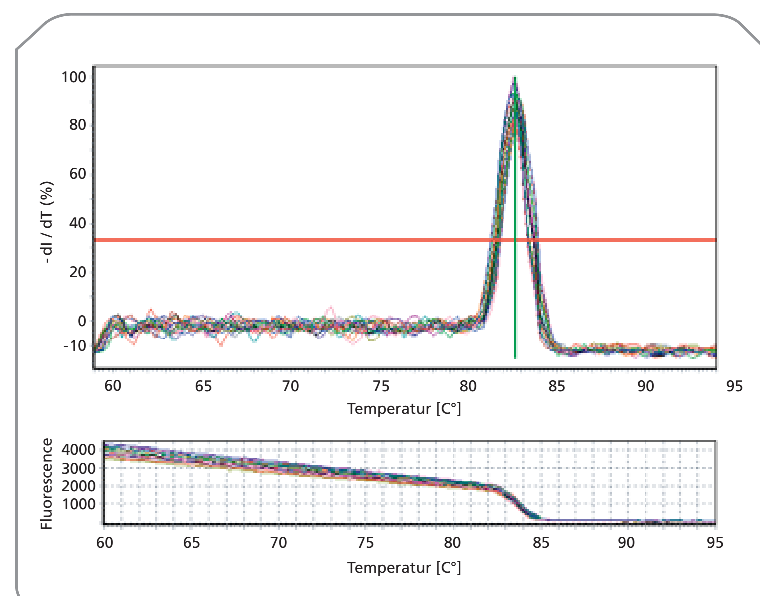


Fig. 14 | Melting Curve Analysis of reaction.

Easy set-up ensuring flexibility with ready-to-use master mix and choice of ROX for SYBR Green or sequence-specific probe detection

For easy and fast handling, all components of the RealMasterMix SYBR ROX are provided in the mastermix.

Separate tubes of 20x SYBR Solution and 20x Probe Enhancer Solution provide flexibility for both SYBR and sequence-specific probe-based assays. When only SYBR Green detection will be performed, addition of the 20 x SYBR Solution to the master mix is recommended. With or without SYBR solution, the RealMasterMix SYBR ROX is stable at 4°C for at least 3 months when stored properly. Repeated freezing and thawing of the solution is eliminated, saving time. An internal reference dye, ROX is included in the RealMaster SYBR ROX buffer for optional reference-dye normalization.

Kit content

	RealMasterMix SYBR ROX 2.5x	
Components/Reactions	**200 Rxns***	**2000 Rxns* = 10 x 200 Rxns**
2.5x RealMasterMix SYBR ROX (0.05 U/µl HotMaster *Taq* DNA Polymerase, 10 mM Magnesium Acetate (4.0 mM final), 1.0 mM dNTPs with dUTP (0.4 mM final)	4 x 1.0 ml	40 x 1.0 ml
20x SYBR Solution	500 µl	10 x 500 µl
20x Probe Enhancer Solution	500 µl	10 x 500 µl
Ref. No.	**2200800**	**2200810**

* 1 reaction = 50 µl

Stop RNase™ Inhibitor

Inhibition of ribonucleases by non-covalent binding of an extremely potent protein

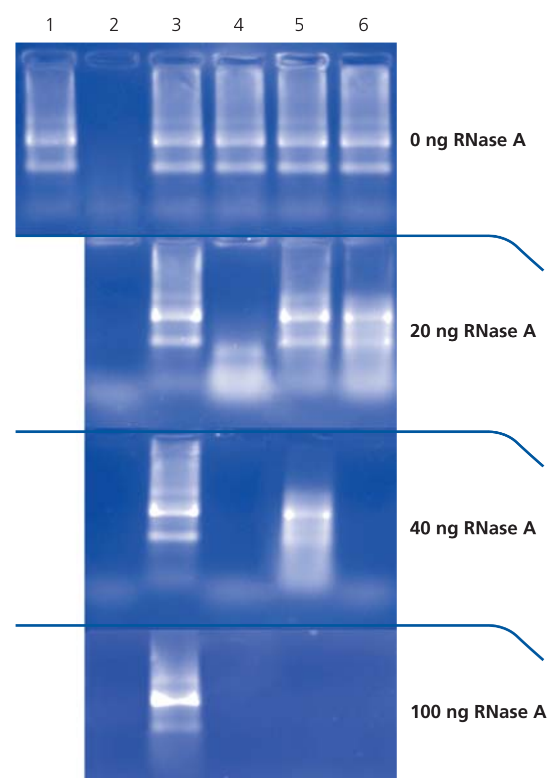
- ⇨ Prevents RNA degradation by inhibiting > 90% of RNase activitiy in a variety of applications
- ⇨ Stable over a broad range of pH, DTT concentrations and temperatures

Prevents RNA degradation by inhibition > 90% of RNase activitiy in a variety of applications

Stop RNase Inhibitor is a protein of non-human origin that binds non-covalently and inhibits a variety of RNase A-type ribonucleases as Human Placental RNase Inhibitor (HPRI), including RNases A, B and C. Stop RNase Inhibitor reduces > 90% of RNAse activity under conditions where HPRI inhibit only 50%.

Inhibition	No Inhibition	
RNase A	RNase T_1	RNase U_1
RNase B	RNase T_2	RNase U_2
RNase C	RNase H	RNase CL_3

Stop RNase Inhibitor can be used in cDNA synthesis, in vitro transcription using the SP6, T7 and HeLa Cell extract systems, and in vitro translations using rabbit reticulocyte lysates and wheat germ extracts. Additionally, Stop RNase Inhibitor prevents RNA degradation in cell extracts under non-denaturing conditions and can be used in first-strand synthesis prior to PCR and in the RT-PCR of RNA isolated from single cells. Stop RNase Inhibitor was used in gel retardation assays, the isolation of an RNA binding protein by oligonucleotide affinity purification, nuclear run-on transcription assays as well as in vitro transcription in S. cerevisiae whole cell extracts, and in polysome distribution analyses at +4°C. The provided concentration is 30 units/µl and 30 units of the Stop RNA Inhibitor are required for a 30 µl sample.



1 Positive Control, No RNase Inhibitor
2 Negative Control, No RNase Inhibitor
3 Stop RNAse Inhibitor
4 Supplier L
5 Supplier I
6 Supplier P

Fig. 15 | RNA Protection Assay with increasing amounts of RNAse Inhibitor from different suppliers.

Kit content

	Stop RNase Inhibitor	
Compontents/Units	**7500 U**	**15000 U**
Stop RNase Inhibitor Solution	1 x 250 µl	2 x 250 µl
Ref. No.	**2500100**	**2500110**

Deoxynucleotide Mix

Ready-to-use, high quality mix of dATP, dCTP, dGTP and dTTP

The Deoxynucleotide Mix is a ready-to-use mix of dATP, dCTP, dGTP and dTTP at concentrations of 10 mM. The dNTPs are in the form of sodium salts (pH 7.0). Nucleotide purity (at least 98%) is confirmed by HPLC and functional PCR tests.
The Deoxynucleotide Mix is suitable for use in all standard and all sensitive PCR techniques, such as long and multiplex PCR as well as RT-PCR.

Amount of Deoxynucleotide Mix (µl) for standard concentration of 0.2 mM each in the appropriate PCR volume:

PCR volume (µl)	20	25	50	100
dNTP Mix (µl)	0.4	0.5	1.0	2.0

Kit content

	Deoxynucleotide Mix -10 mM	
dNTP-Mix – 10 mM each dNTP	1 x 200 µl	1 x 1000 µl
Ref. No.	**2201200**	**2201210**

Deoxynucleotide Set

Separate solutions for high quality nucleotides dATP, dCTP, dGTP and dTTP

The Deoxynucleotide Set provides separate solutions of dATP, dCTP, dGTP and dTTP at concentrations of 100 mM. The dNTPs are in the form of sodium salts (pH 7.0). Nucleotide purity (at least 98%) is confirmed by HPLC and functional PCR tests.
The Deoxynucleotide Mix is suitable for use in all standard and all sensitive PCR techniques, including long and multiplex PCR, RT-PCR, DNA labeling and sequencing applications.

Kit content

	Deoxynucleotide Set	
100 mM of each dNTP	4 x 100 µl	4 x 250 µl
Ref. No.	**2201220**	**2201230**

10x *Taq* Buffer with 15 mM Magnesium

Standard Mg^{2+} containing reaction buffer with self adjusting Mg^{2+} buffer technology

The 10x *Taq* Buffer with 15 mM Magnesium is the standard reaction buffer for all PCR applications. The buffer composition is 500 mM KCl, 100 mM Tris-HCl, pH 8.3 (at 25°C) and contains 15 mM Magnesium.

Kit content

	10x *Taq* Buffer with 15 mM Magnesium
10x *Taq* Buffer with 15 mM Magnesium	1 x 1.8 ml
Ref. No.	**2201240**

5x *Taq*Master PCR Enhancer

Buffer additive for improve thermostability and processivity of *Taq*

*Taq*Master PCR Enhancer is a buffer additive that improves thermostability (enzyme half-life) and processivity of *Taq* polymerase by stabilizing the enzyme during PCR. The *Taq* enzyme becomes less sensitive to exogenous PCR-inhibiting contamination (e.g., humic acid from soil samples, blood compounds, etc.). In contrast to other PCR-enhancing additives, *Taq*Master is chemically stable and inert. It's use does not affect primer annealing or template denaturation, allowing easy adaptation of existing protocols. Increased yields and reproducible results are obtained when amplifying impure samples or GC-rich templates with *Taq*Master PCR Enhancer.

Kit content

	5x *Taq*Master PCR Enhancer
5x *Taq*Master PCR Enhancer	1 x 1.7 ml
Ref. No.	**2201250**

GelElute Extraction Kit

For batch purification of DNA fragments from gels and solutions

- Efficient recovery – DNA from 40 bp to 50 kb without shearing
- No interference of downstream application by NaI
- Versatile use – extraction from agraose and polyarcylamide gels
- Optimal use – pH indicator in binding buffer for maximize adsorption

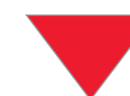
Efficient recovery of DNA from 40 bp to 50 kb without shearing

Purification of DNA fragments with the GelElute system is based on solubilization of agarose and selective adsorption of nucleic acids to the GelElute silica-gel particles in the presence of chaotropic salt.

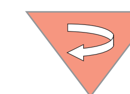
GelElute silica-gel particles are added to the solubilized gel slice (see procedure) and collected with a brief centrifugation step. After washing, the pure DNA fragment is eluted in 20 µl of Tris buffer or water. GelElute particles ensure efficient recovery without shearing, even for large DNA fragments.

GelElute Principle

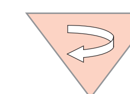
Solubilized gel slide + GelElute Particles



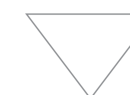
Bind



Wash



Elute



Pure DNA Fragment in 30 minutes

No interference of downstream application by NaI

Optimized buffers permit DNA recovery without sodium iodide, which is difficult to remove from DNA samples and can affect subsequent reactions. Quantities of DNA from 10 ng to 10 µg are recovered efficiently (see figure 16 page 28). The versatile batch procedure can be easily scaled up for preparative purposes for up to 15 µg binding capacity using 30 µl GelElute suspension.

Versatile use - extraction from agraose and polyarcylamide gels

GelElute separates DNA from salts, agarose, polyacrylamide, dyes, proteins, and nucleotides without phenol extraction or ethanol precipitation. GelElute is effective for any type of agarose in either TAE or TBE buffers.

DNA purified with the GelElute system can be used directly in most applications, including:

- Restriction digestion
- Ligation
- Labeling
- PCR

pH indicator in binding buffer for maximize adsorption

The solubilization and binding buffer used with the GelElute system contains a pH indicator, allowing easy visual determination of optimal pH for DNA adsorption (pH ≤ 7.5). The colored dye also allows

easy visualization of any unsolubilized agarose present in the binding mixture, ensuring complete solubilization for maximum yield.
An incorrect binding-mixture pH can arise if the agarose gel electrophoresis buffer was frequently used or incorrectly prepared. In this case the pH can be easily adjusted by addition of 10 µl 3 M sodium acetate, pH 5.0.

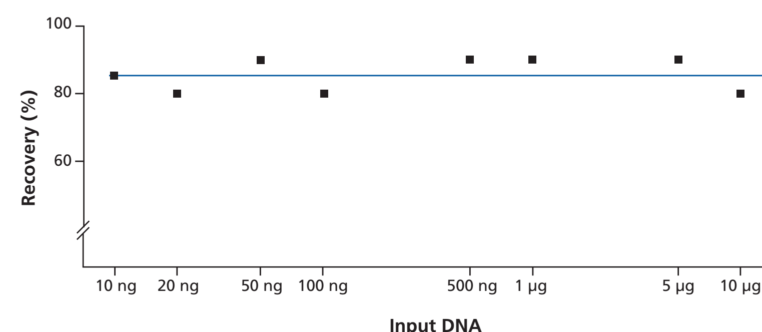


Fig. 16 | Recovery of various amounts of a 2.9 kb DNA fragment from 1% agarose gels using the GelElute Extraction Kit.

Kit content

	GelElute Extraction Kit		GelElute Extraction Suspension – 1.5 ml
Component/Preparations	**150 Preps**	**500 Preps**	**–**
GelElute Suspension	3 x 0.5 ml	5 x 1.0 ml	1 x 1.5 ml
Buffer GX1 (with pH Indicator)	2 x 100 ml	6 x 100 ml	–
Buffer GE	2 x 20 ml	6 x 20 ml	–
Ref. No.	**2300400**	**2300410**	**2300420**

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