Tune into perfect PCR

3rd Edition

PCR Enzyme Guide

Highly Sensitive and Efficient DNA Amplification

A Meridian Life Science® Company

Bioline the PCR Enzyme Company

For the last 20 years, Bioline has been engaged in the development and manufacturing of one of the broadest portfolios of premium quality PCR enzymes, for a wide range of applications. Our commitment to this area is demonstrated with the introduction of the MyFi™, RANGER and MyTaq™ product range, a new generation of highest performance DNA polymerases.

This guide aims to help you to choose the polymerase best suited to your needs. Each DNA polymerase has different characteristics and to achieve optimal results, it is important for the user to choose the polymerase most suited to their application. For your convenience and to achieve optimal PCR, many of our most popular PCR enzymes are also available in practical, ready-to-use 2x mastermixes, which contain polymerase, dNTPs, $MgCl_2$, and additional additives. A polymerase selection table is provided to facilitate your choice.

	Properties	Template Length	Hot-Start	Proofreading	High Processivity	**Available Mixes**	Mixes	**Applications**	Long Range PCR (over 10kb)	High Specificity Assays	Blunt End Cloning	TA Cloning	GC-Rich Templates	Low Copy Templates	Site-Directed Mutagenesis	High-Fidelity PCR
MyTaq™ HS		Up to 5kb	✓				✓			✓		✓	✓	✓		
IMMOLASE™		Up to 5kb	✓							✓		✓		○		
MyFi™		Up to 10kb	✓		✓		✓			✓		✓	○	✓		○
VELOCITY		Up to 10kb		✓	✓						✓		○	○	✓	✓
ACCUZYME™		Up to 5kb		✓			✓				✓				✓	✓
RANGER		Up to 25kb	✓	○	✓		✓		✓	✓		✓	○	✓		○
BIO-X-ACT™ Short		Up to 8kb			✓		✓					✓	○			○
MyTaq™		Up to 5kb					✓					✓	✓			
Mango*Taq*™		Up to 5kb					✓					✓				
BIOTAQ™		Up to 5kb					✓					✓				

	Crude Sample PCR	Fast PCR	Direct Gel Loading	Colony PCR
	○	✓	✓	✓
	○	✓		
	○	○		
	✓	✓		
	○	✓	✓	✓
		○	✓	
		○	✓	

✓ Recommended
○ Suitable

Contents

Hot-Start DNA Polymerases	**04**
MyTaq™ HS DNA Polymerase	05
MyTaq™ HS Red DNA Polymerase	05
MyTaq™ HS Mix	05
MyTaq™ HS Red Mix	05
IMMOLASE™ DNA Polymerase	06
MyFi™ DNA Polymerase	07
MyFi™ Mix	07
High-Fidelity DNA Polymerases	**08**
VELOCITY DNA Polymerase	09
VELOCITY PCR Kit	10
PCR Tailing Mix	10
ACCUZYME™ DNA Polymerase	11
ACCUZYME™ Mix	11
DNA Polymerases for Specialized Applications	**12**
RANGER DNA Polymerase	12
RANGER Mix	12
BIO-X-ACT™ Short DNA Polymerase	13
BIO-X-ACT™ Short DNA Mix	13
DNA Polymerases for Routine Applications	**14**
MyTaq™ DNA Polymerase	15
MyTaq™ Red DNA Polymerase	15
MyTaq™ Mix	15
MyTaq™ Red Mix	15
Mango*Taq*™ DNA Polymerase	16
MangoMix™	16
BIOTAQ™ DNA Polymerase	17
BIOTAQ™ PCR Kit	17
One-Step RT-PCR	**18**
MyTaq™ One-Step RT-PCR Kit	18
DNA Polymerase Reagents	**18**
dNTP Mixes	18
dNTP Sets	18
General Considerations and Optimization	**19**
Troubleshooting	**21**
Bulk, Custom and OEM Services	**22**
Ordering Information	**23**

Hot-Start DNA Polymerases

Hot-start PCR is a technique used to reduce nonspecific amplification when assembling amplification reactions at room temperature. During assembly at room temperatures, PCR primers can anneal to template sequences that are not perfectly complementary. Since thermostable DNA polymerases have some activity even at room temperature, the polymerase can extend mis-annealed primers. This newly synthesized region then acts as a template for primer extension and synthesis of undesired amplification products. If however the reaction is heated to temperatures above 65°C before polymerization begins, the stringency of primer annealing is increased and synthesis of undesired PCR products is avoided (1).

Hot-start PCR can also reduce the amount of primer-dimer (2) by increasing the stringency of primer annealing. At lower temperatures, PCR primers can anneal to each other via complementary regions and the DNA polymerase can extend the annealed primers to produce primer dimer. This can often appear as a diffuse band of approximately 50–100bp on an ethidium bromide-stained gel. The formation of non-specific products and primer-dimer can compete for reagent availability with amplification of the desired product. Thus, hot-start PCR can improve the yield of specific PCR products.

In a manual reaction, hot-start can be achieved by omitting a vital component of the reaction (enzyme, primers, template or dNTPs) from the PCR mix. The reaction tube is then heated to the denaturation temperature before the missing component is added. It is time-consuming and impractical however, to add components to the tubes or plates in the thermal cycler, particularly when large numbers of reactions are being carried out. This is why alternative hot-start methods were developed, the two most commonly used are chemical-modification and antibody-mediated.

Chemically-modified *Taq* hot-start methods use a *Taq* polymerase which has been modified with the addition of a heat labile blocking group. The addition of a heat-labile group inactivates the enzyme at room temperatures. Incubation at 95°C for 10 minutes results in removal of the group and activation of the enzyme (3-6). One disadvantage with this method is that a long pre-incubation step is needed prior to cycling in order to activate the enzyme.

Antibody-mediated, relies on a *Taq* antibody which is bound to *Taq* DNA polymerase. The antibody-bound *Taq* complex is inactive until the initial denaturation step, of 95°C for 1-2 minutes, when the antibody is heat-denatured, releasing it from the enzyme and restoring full activity to *Taq*. (7,8).

MyTaq™ HS DNA Polymerase and Mix

Best in class

FEATURES

- **New generation of antibody-based hot-start polymerase**
- **Highest specificity and superior performance**
- **Novel buffer system, including ultra-pure dNTPs and $MgCl_2$**
- **Red dye for direct gel loading**
- **Convenient all-in-one mastermix**

APPLICATIONS

- **Fast PCR reactions**
- **Assays with prolonged reaction setup on the bench or liquid handling**
- **Colony PCR**
- **Multiplexing**
- **Specific amplification of difficult templates (GC rich)**

PRODUCT	PACK SIZE	PRESENTATION	CAT NO.
MyTaq HS DNA Polymerase	250 Units	1 x 50µl	BIO-21111
	1000 Units	1 x 200µl	BIO-21112
	2500 Units	2 x 250µl	BIO-21113
MyTaq HS Red DNA Polymerase	250 Units	1 x 50µl	BIO-21114
	1000 Units	1 x 200µl	BIO-21115
	2500 Units	2 x 250µl	BIO-21116
MyTaq HS Mix	200 Reactions	4 x 1.25ml	BIO-25045
	500 Reactions	10 x 1.25ml	BIO-25046
MyTaq HS Red Mix	200 Reactions	4 x 1.25ml	BIO-25047
	500 Reactions	10 x 1.25ml	BIO-25048

MyTaq™ HS is a new generation of very high performance, antibody-mediated hot-start PCR products, developed by Bioline and designed to give outstanding results with complex genomic DNA templates. MyTaq HS uses the latest technology in PCR enzyme design, engineered to increase affinity for DNA, so resulting in significant improvements in yield, sensitivity and speed. The product also has the added convenience of room temperature reaction assembly, so avoiding non-specific amplification and primer-dimer formation.

The enzyme is supplied with an industry-leading novel buffer system, consisting of a proprietary formulation containing dNTPs, $MgCl_2$ and enhancers at optimal concentrations, removing the need for optimization and giving superior amplification (fig. 1).

MyTaq HS Mix is a ready-to-use 2x mix for fast, highly-specific, hot-start PCR. The advanced formulation of MyTaq HS Mix allows fast cycling conditions to be used, greatly reducing the reaction time without compromising PCR specificity and yield (fig. 2).

MyTaq HS is also available with an inert red dye. Following PCR, samples can be loaded directly onto the agarose gel without the need for a loading buffer, since the mix is of sufficiently high density to sink to the bottom of the well.

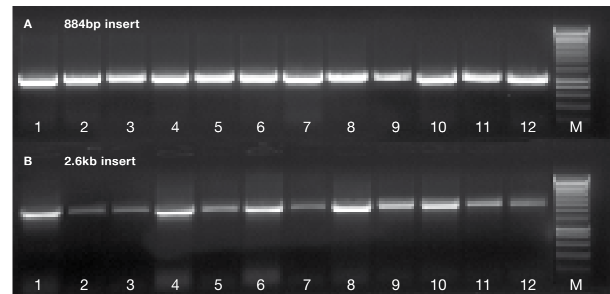


Fig. 1 Robustness of MyTaq HS in Colony PCR
E.coli transformed with M13 carrying A) a 2.6 kb or B) an 884 bp insert were plated out. 12 colonies were picked with tooth-picks and transferred directly into MyTaq buffer and amplified with MyTaq HS. Reaction conditions were 95°C for 3 min, followed by 35 cycles of 95°C for 15s, 60°C for 15s and 72°C for 2mins. Marker is HyperLadder I (M) (Cat No. BIO-33025). The results illustrate that MyTaq HS is robust enough to amplify colonies directly from a plate.

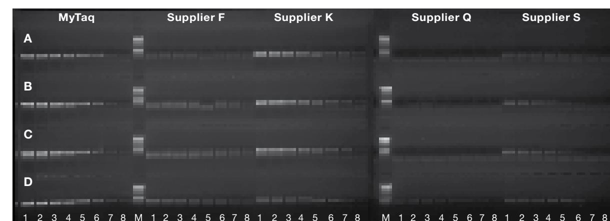


Fig. 2 Fast amplification (26.3 minutes) was carried out on a range of human genomic genes
A) A 340bp and B) a 450bp fragment of the *myc* gene, C) a 525bp fragment of the *EGFR* gene and D) a 530bp fragment of the *AGRI1* gene were amplified with MyTaq HS and the results were compared with amplifications with hot-start DNA polymerases from other suppliers. The process used a serial dilution of human genomic DNA (100ng, 33ng, 10ng, 4ng, 1ng, 33pg, 10pg and 3pg genomic DNA, lanes 1-8 respectively), incubated for 3mins at 95°C followed by 35 cycles of 15s at 95°C, 55°C and 72°C. Marker is HyperLadder I (M) (Cat No. BIO-33025). MyTaq HS performed well across all four human genes.

Hot-Start DNA Polymerases References:

1. Chou, Q., *et al. Nucleic Acids Res.* **20(7)**, 1717–1723 (1992).
2. Handyside, A., *et al. Nature* **344**, 768-770 (1990).
3. Dixon, H. & Perham, R. *Biochem. J.* **109**, 312-314 (1968).
4. Shetty, J. & Kinsella, J. *Biochem. J.* **191**, 269-272 (1980).
5. Nieto, M. & Palacian, E. *Biochimica et Biophysica Acta* **749**, 204-210 (1989).
6. Atassi, M. *et al. Methods in Enzymolo.* **25**, 546-553 197 (1972).
7. Scalice, E. *et al. J. Immunol. Methods* **172**, 147–163 (1994).
8. Saiki, R. *et al. Science* ***230***, 1350-1354 (1985).

IMMOLASE™ DNA Polymerase

Fire up your PCR

PRODUCT	PACK SIZE	PRESENTATION	CAT NO.
IMMOLASE DNA Polymerase	250 Units	1 x 50µl	BIO-21046
	500 Units	1 x 100µl	BIO-21047
	5000 Units	10 x 100µl	BIO-21048

FEATURES

- **Heat-activated thermostable DNA polymerase**
- **Outstanding and robust performance**
- **Excellent yield in quantitative assays**
- **Convenient setup at room temperature**
- **Leaves ‘A’ overhang**

APPLICATIONS

- **Ultra-high specificity for multiplex reactions**
- **Products suitable for TA cloning**
- **Low-copy number templates**
- **Multiplex PCR reactions**

IMMOLASE™ is a heat-activated thermostable DNA polymerase. IMMOLASE provides extremely high yield (fig. 1), improved specificity as compared to standard polymerases and can eliminate the presence of non-specifics, such as primer-dimers and mis-primed products. IMMOLASE is inactive at room temperature and therefore prior to PCR cycling, requires activation by heat treatment for 10 minutes (fig. 2). This enables flexibility in reaction setup, including premixing of PCR reagents at room temperature. Subsequently, the reaction can be handled according to the user’s existing protocols for thermostable DNA Polymerases.

Specificity and performance of IMMOLASE can be further improved with the use of 2x PolyMate Additive (BIO-37041, not supplied), which is designed for GC- or AT-rich DNA, “dirty” templates or sequences with a high level of secondary structure.

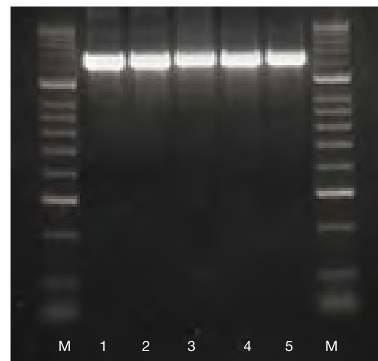


Fig. 1 Extremely high yield amplification
A 1.4Kb mouse *rn18s* gene fragment was amplified with 2.5 Units of IMMOLASE DNA Polymerase (lanes 1-5). The *rn18s* fragment was amplified from 100ng of mouse genomic DNA. The PCR was performed in 50µl reaction mixtures containing 1.5mM $MgCl_2$. HyperLadder II (M). Extremely high yield is achieved with every replicate.

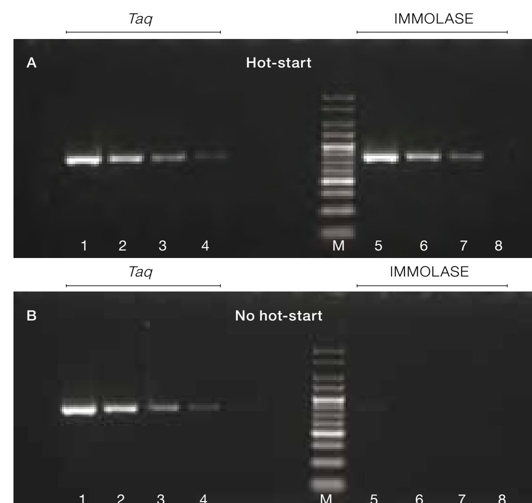


Fig. 2 Illustration of IMMOLASE Heat-Activation
Two experiments were performed, one with hot-start (A) and one without hot-start (B). A 125bp DNA fragment from plasmid pGEM was amplified with 1.0 Unit of *Taq* (lanes 1-4) and 1.0 Unit of IMMOLASE (lanes 5-8). The pGEM fragment was amplified from 0.25ng plasmid DNA (pGEM) followed by 2-fold serial dilutions in 50µl reactions containing 1.5mM $MgCl_2$. HyperLadder V (M). *Taq* exhibited activity in both tests, whereas IMMOLASE only exhibited activity following a hot-start step.

NEW

MyFi™ DNA Polymerase and Mix

Higher fidelity with enhanced specificity

PRODUCT	PACK SIZE	PRESENTATION	CAT NO.
MyFi DNA Polymerase	250 Units	1 x 125µl	BIO-21117
	500 Units	1 x 250µl	BIO-21118
	2500 Units	2 x 625µl	BIO-21119
MyFi Mix	100 Reactions	2 x 1.25ml	BIO-25049
	500 Reactions	10 x 1.25ml	BIO-25050

FEATURES

- High-fidelity, specificity and sensitivity
- Novel antibody-based hot-start polymerase
- Amplifies fragments up to 10kb
- Industry-leading novel buffer system
- Available as an all-in-one master mix

APPLICATIONS

- PCR requiring high specificity combined with high-fidelity
- Suitable for TA cloning
- Suited to site directed mutagenesis
- Ideal for low copy PCR assays

MyFi™ is a novel, antibody-mediated, hot-start enzyme with unique properties that offers 3.5x higher fidelity than native *Taq* and enhanced specificity. The polymerase is supplied with MyFi Buffer, a highly optimized proprietary formulation, containing ultra-pure dNTPs, $MgCl_2$ and enhancers, specifically formulated and validated to the unique properties of MyFi.

MyFi is ideally suited for difficult PCR amplification of targets with variable lengths up to 10kb, for example, amplification of cDNA libraries, complex genomic fragments, targets with high GC-content (fig. 1) and low-copy assays which require both high processivity and higher fidelity (fig. 2). MyFi has the added convenience of room temperature reaction assembly, to avoid non-specific amplification such as primer-dimer formation.

MyFi Mix is a ready-to-use 2x mix, containing all the reagents (including enhancers and stabilizers) necessary for trouble-free PCR reaction set-up. The unique MyFi Mix formulation, supplied in a convenient single tube, reduces the number of pipetting steps and improves efficiency, throughout and reproducibility (fig. 2).

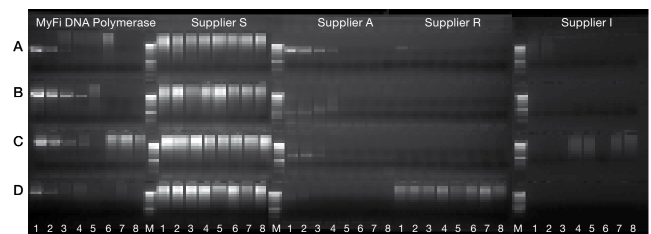


Fig. 1 Amplification of complex DNA up to 10kb
A) A 3.9kb fragment of α-1-antitrypsin (*AT-R3*) gene, B) a 7.0kb fragment, C) a 9.0kb fragment and D) a 10.0kb fragment (respectively) of human (β-globin) *HbG* gene, were amplified using MyFi DNA Polymerase and the results were compared with amplifications using high-fidelity hot-start DNA polymerases from other suppliers. The process used a serial dilution of human genomic DNA (5ng, 1ng, 200pg, 40pg, 8pg, 1.6pg, 0.32pg and 0pg, lanes 1-8 respectively), incubated for 3 min at 95ºC (or according to the manufacturer's protocol) followed by 35 cycles of 30s at 95ºC, 30s at 60ºC and 5min at 72ºC respectively. Marker is HyperLadder I (M). The results illustrate that MyFi can be used to amplify products up to 10kb, unlike many of the competing high-fidelity hot-start DNA polymerases tested.

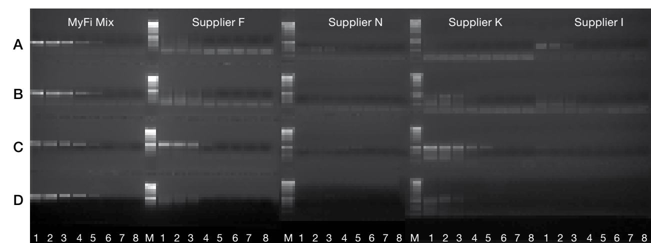
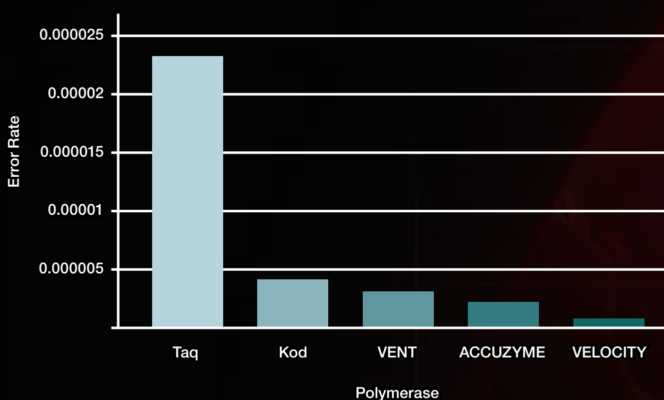


Fig. 2 Efficiency and sensitivity of high-fidelity polymerase mixes
A) A 525bp fragment of human epidermal growth factor receptor (*EGFR*) gene, B) a 750bp fragment of translation factor p64 (*myc*) gene, C) a 900bp fragment of angiotensin II receptor type I (*AGTR1*) gene, D) a 1.2kb fragment of *EGFR* gene, were amplified using MyFi Mix and the results were compared with amplifications using high-fidelity hot-start DNA polymerases from other suppliers. The process used a serial dilution of human genomic DNA (5ng, 1ng, 200pg, 40pg, 8pg, 1.6pg, 0.32pg and 0pg human genomic DNA, lanes 1-8 respectively), incubated for 3 min at 95ºC (or according to the manufacturer's protocol) followed by 35 cycles of 15s at 95ºC, 15s at 57ºC and 15s at 72ºC. Marker is HyperLadder I (M). The results illustrate that MyFi Mix out-performed alternative suppliers of high-fidelity mixes on account of higher efficiency and sensitivity over a wide range of sizes.

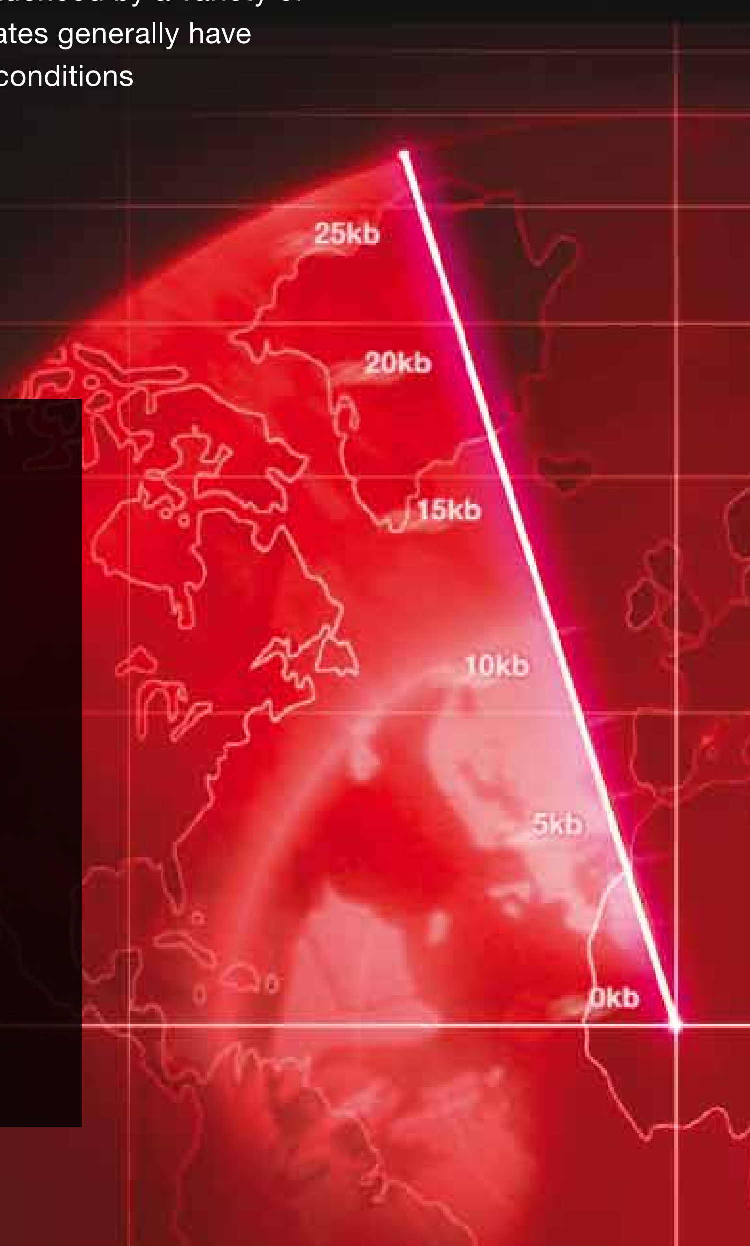
High-Fidelity DNA Polymerases

High-fidelity DNA polymerases are extremely valuable for minimizing the introduction of amplification errors in products that will be cloned, sequenced and expressed. Significant time and effort is saved by using high-fidelity polymerases that eliminate the need for downstream error-correction steps and minimize the number of clones that need to be sequenced in order to obtain error-free constructs. Moreover, the use of high-fidelity polymerases is essential when analyzing very small amounts of template DNA or rare molecules in heterogeneous populations (1). Amplifications employing small amounts of template DNA are especially prone to high mutant frequencies due to PCR-generated errors in early cycles. When analyzing rare sequences, such as allelic polymorphisms in individual mRNA transcripts, allelic stages of single cells, or rare mutations in human cells (2), it is essential that polymerase-generated errors are minimized to prevent masking of rare DNA sequences.

PCR fidelity is largely determined by the intrinsic error rate of a DNA polymerase under the reaction conditions employed. Enzyme fidelity can by influenced by a variety of factors, including template sequence (i.e. GC-rich templates generally have increased error rates), cycling parameters, and reaction conditions (i.e. pH, Mg^{2+}, dNTP concentration). The importance of proofreading (a 3'→5' exonuclease activity) is evident in comparisons of base substitution error rates between non-proofreading (10^{-2} to $>10^{-6}$ errors per base) and proofreading (10^{-6} to 10^{-7} errors per base) DNA polymerases (3).



Standard *Taq* DNA polymerases are suitable for a number of PCR applications and are still considered by many to be the industry standard, however, the performance of these polymerases is limited in more challenging applications. Proofreading DNA polymerases often display low processivity resulting in low yield, reduced product length and difficulties in optimization. Bioline's high-fidelity polymerases (VELOCITY, ACCUZYME and MyFi) have been developed to offer very high-fidelity as well as excellent amplification efficiency and extended product length.



VELOCITY DNA Polymerase

Perfect for GC-rich templates

PRODUCT	PACK SIZE	PRESENTATION	CAT NO.
VELOCITY DNA Polymerase	250 Units	1 x 125µl	BIO-21098
	500 Units	1 x 250µl	BIO-21099
VELOCITY PCR Kit	20 Reactions	250 Units	BIO-21104
PCR Tailing Mix	50 Reactions	1 x 250µl	BIO-21103

FEATURES

- **High-speed, high-fidelity DNA polymerase**
- **High-processivity**
- **Fast amplification**
- **Shorter PCR runs for longer templates**
- **Robust, requires minimal optimization of the reaction**

APPLICATIONS

- **GC-rich templates**
- **Cloning techniques where high-fidelity is desirable**
- **Blunt-end cloning**
- **Amplification of difficult templates**
- **Site directed mutagenesis**

VELOCITY DNA Polymerase is an ultra fast thermostable enzyme possessing 3'→5' proofreading exonuclease activity. VELOCITY delivers outstanding PCR yield with exceptional fidelity, even from low template concentrations (fig. 1). It also has high processivity, resulting in shorter extension times, higher yield and the ability to amplify long templates in a fraction of the time. Furthermore, the polymerase offers robust and reliable yields, even in assays in which PCR conditions are compromised with impurities or in complex assays (fig. 2), enabling the polymerase to be used with minimal optimization.

VELOCITY provides both high-fidelity coupled with an extremely low error-rate of 4.4 x 10^{-7} and inherently high processivity, resulting in extension rates as fast as 15s/kb for templates of up to 5kb and 30s/kb for templates longer than 5kb. Reduction in PCR turnaround time makes VELOCITY the ideal choice for users who wish to generate long PCR products with high yield and no mutations.

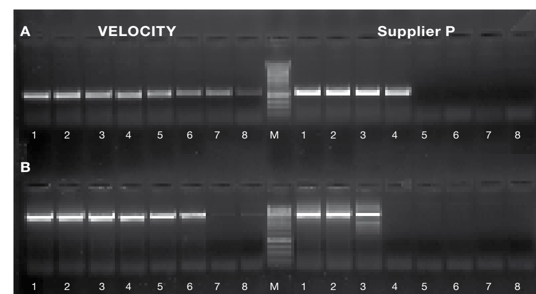


Fig. 1 High-fidelity and high-yield amplification of human genomic DNA
A) a 1kb and B) a 10kb fragment was amplified from 10ng, 2ng, 400pg, 80ng, 16pg, 3.2pg, 0.6pg and 0.1pg (lanes 1-8 respectively) of human genomic DNA with VELOCITY and supplier P. Reactions were incubated at for 98°C for 2 min followed by 30 cycles at 98°C for 30s, 55°C, for 30s, and 72°C for 1 or 10 min. HyperLadder I (M). The results illustrate that VELOCITY delivers products even at very low template concentrations.

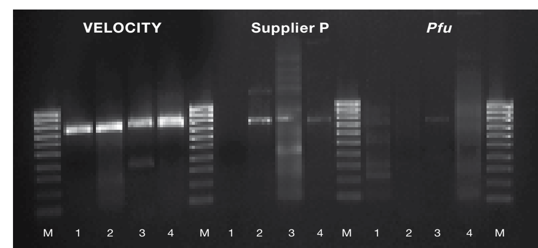


Fig. 2 Amplification of GC-rich DNA fragments from human genomic DNA
VELOCITY, a competitor polymerase (P) and wild-type *Pfu* were compared. Lanes 1–4 are a 728bp fragment of the *GP150* gene (76.9% GC), a 724bp fragment of the *MRGRE* gene (68% GC), a 723bp fragment of the *NM_022372.3* gene (66.9% GC) and a 788bp fragment of the *NM_033178.2* gene (70.9% GC) respectively. PCR was performed in 50µl reaction mixes and 5µl was run on a 1.5% TAE agarose gel. HyperLadder IV (M). The results illustrate that VELOCITY is reliable even with GC rich templates.

High-Fidelity DNA Polymerases References

1. Cha, R. & Thilly, W. PCR Primer: A Laboratory Manual. *Cold Spring Harbor Laboratory Press* (1995).
2. Andre, P., *et al. Genome Res.* **7**, 843-852 (1997).
3. Cline, J., *et al.. Nucleic Acids Res.* **24**(7), 3456-3551 (1996).

VELOCITY PCR Kit

The right clone first time

FEATURES

- **High-speed, high-fidelity DNA polymerase**
- **Robust performance with problematic GC and AT rich targets**
- **PCR Tailing Mix to improve the efficiency of cloning**

APPLICATIONS

- **Cloning techniques where high-fidelity is desirable**

To enhance the cloning efficiency of DNA amplified with VELOCITY, Bioline has developed a new VELOCITY PCR Kit. The kit contains VELOCITY DNA polymerase to generate error free PCR products and a PCR Tailing Mix to add a 3'-A overhang, to facilitate TA cloning.

VELOCITY PCR products are blunt-ended due to the 3'→5' exonuclease activity of the polymerase which removes 3'-A overhangs. A 3'-A overhang is useful, however, as it facilitates more efficient cloning into plasmid vectors and help prevent insert-to-insert ligation, eliminating possible tandem inserts. In order to generate a 3'-A overhang, the kit also contains a PCR Tailing Mix. This uses a uniquely blended *Taq* polymerase to add a single Adenine base as well as an exonuclease inhibitor to reduce the 3'→5' exonuclease activity of VELOCITY, thus eliminating the need for purification of the PCR products prior to addition of the overhang.

5µl of the PCR Tailing Mix is added directly to 50µl of the amplified product, without the need for prior purification (fig. 1), and incubated at 72°C for 5 minutes.

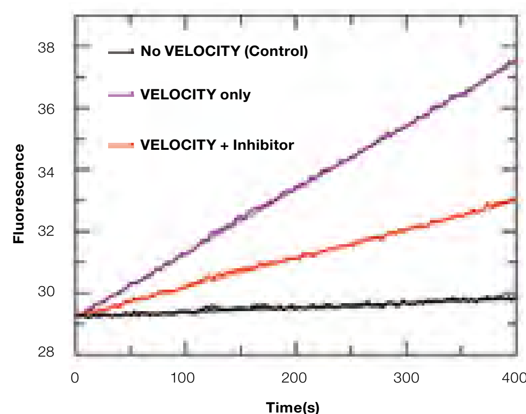


Fig. 1 VELOCITY Inhibition by PCR Tailing Mix
Taq overextension is negated by the exonuclease activity from the VELOCITY DNA polymerase, reducing ligation/cloning efficiency. The *Taq* 3'-A overextension reaction contains an exonuclease inhibitor, which reduces the 3'→5' exonuclease activity of VELOCITY by more than 2-fold. Subsequently, it is not necessary to either clean-up the PCR product, before adding *Taq*, or maintain the post-PCR modified product on ice prior to ligation into a cloning vector.

PCR Tailing Mix

Cloning blunt ended amplicons

FEATURES

- **Fast addition of 3'-A overhang to blunt ended DNA fragments**
- **Uses exonuclease inhibitor so no post PCR purification is required**

APPLICATIONS

- **TA cloning techniques where high-fidelity is desirable**

Proofreading DNA Polymerases provide high-fidelity, however the resulting PCR product is blunt-ended, because the proofreading polymerases possess a 3'→5' exonuclease activity that removes the 3'-A overhangs normally added by *Taq* DNA polymerase. These 3'-A overhangs are useful for efficient cloning of these fragments into suitable plasmids (TA cloning), and effectively prevent insert-to-insert ligation, eliminating possible tandem inserts. The PCR Tailing Mix uses *Taq* DNA polymerase and an exonuclease inhibitor to add a single terminal A, whilst inhibiting the 3'→5' exonuclease activity of the proofreading DNA polymerase.

ACCUZYME™ DNA Polymerase and Mix

Right on target

PRODUCT	PACK SIZE	PRESENTATION	CAT NO.
ACCUZYME DNA Polymerase	250 Units	1 x 100µl	BIO-21051
	500 Units	1 x 200µl	BIO-21052
ACCUZYME Mix	100 Reactions	2 x 1.25ml	BIO-25027
	500 Reactions	10 x 1.25ml	BIO-25028

FEATURES

- Very high PCR sensitivity, suited to low-copy assays
- Intrinsic high processivity
- High-fidelity
- Amplifies fragments up to 5kb
- Available as a convenient pre-mixed, pre-optimized solution (ACCUZYME Mix)

APPLICATIONS

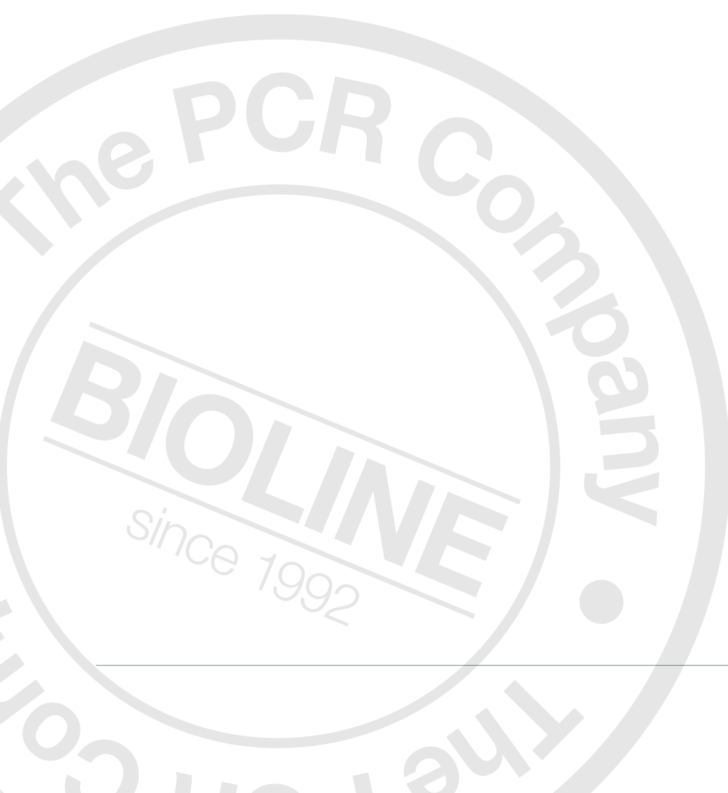
- High-fidelity PCR ideal for subsequent cloning
- Blunt-end cloning
- Site directed mutagenesis

ACCUZYME™ is a thermostable enzyme possessing 5'→3' DNA polymerase and 3'→5' proofreading exonuclease activities, offering high-fidelity, even with demanding applications (fig. 1). ACCUZYME produces blunt-ended amplicons up to 5kb.

ACCUZYME possesses very high PCR sensitivity and is ideally suited to low-copy target amplifications.

ACCUZYME Mix dramatically reduces the time needed to set up reactions, thereby minimizing the risk of contamination. Greater reproducibility is ensured by the reduction in the number of pipetting steps that can lead to pipetting errors.

Fig. 1 High performance with ACCUZYME at low template concentrations
An 800bp fragment was amplified from 500ng, 50ng, 5ng, 0.5ng, 50pg and 5pg (lanes 1-6 respectively) of human genomic DNA with ACCUZYME Mix. Reactions were incubated at for 95°C for 3 min followed by 30 cycles at 95°C for 15s, 55°C, for 15s, and 72°C for 1 min. HyperLadder I (M). The results illustrate that ACCUZYME is highly sensitive even with low template concentrations.



NEW **RANGER** DNA Polymerase and Mix

For long amplicons

PRODUCT	PACK SIZE	PRESENTATION	CAT NO.
RANGER DNA Polymerase	250 Units	1 x 62.50µl	BIO-21121
	500 Units	1 x 125µl	BIO-21122
	2500 Units	2 x 312.50µl	BIO-21123
RANGER Mix	100 Reactions	2 x 1.25ml	BIO-25051
	500 Reactions	10 x 1.25ml	BIO-25052

FEATURES

- **Fast antibody-based hot-start**
- **Novel buffer system, including ultra-pure dNTPs and $MgCl_2$**
- **Higher fidelity than *Taq***
- **Available as a convenient all-in-one master mix**

APPLICATIONS

- **Validated for human genomic DNA up to 25kb**
- **Suitable for TA cloning**

RANGER DNA Polymerase is a hot-start enzyme, possessing 5´→3´ DNA polymerase and 3´→5´ proofreading exonuclease activities, thus offering both high-fidelity and enhanced specificity. The polymerase is supplied with an industry-leading novel buffer system, specifically formulated and validated for the unique properties of RANGER.

RANGER is an easy-to-use high-performance enzyme specifically designed to amplify templates from 10kb or greater with extreme sensitivity (fig. 1). Due to its antibody-based hot-start property, RANGER has the added convenience of room temperature reaction assembly, reducing unwanted non-specific amplification such as primer-dimer formation. This new hot-start enzyme from Bioline is supplied with 5x RANGER Reaction Buffer, a proprietary formulation containing dNTPs, $MgCl_2$ and enhancers at optimal concentrations, providing superior amplification.

RANGER is highly suitable for all PCR applications of long templates, including sequencing, mapping of chromosomal translocation breakpoints and other structural variations, as well as TA cloning.

RANGER Mix is a ready-to-use 2x mix, lacking polymerase activity during the reaction set-up, thus reducing non-specific amplification. The advanced formulation of RANGER Mix enables extreme sensitivity (fig. 2) and increased fidelity. RANGER Mix contains all the reagents necessary for trouble-free PCR reaction set-up. For your convenience, all of the components are supplied in one tube, to reduce the number of pipetting steps and to improve efficiency and reproducibility.

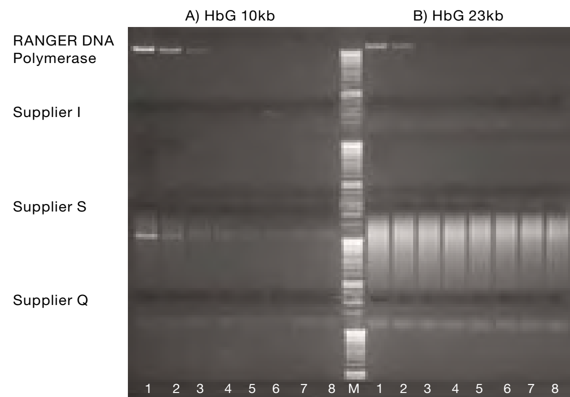


Fig. 1 Amplification of complex DNA greater than 10kb
A) A 10kb fragment and B) a 23kb fragment of human β-globin (*HbG*) gene, were amplified using RANGER Polymerase and the results were compared with amplifications using high-fidelity hot-start DNA polymerases from other suppliers. The process used a serial dilution of human genomic DNA (5ng, 1.6ng, 550pg, 180pg, 60pg, 20pg, 6pg and 0pg human genomic DNA, lanes 1-8 respectively), incubated for 1 min at 95ºC (or according to the manufacturer's protocol) followed by either 30 cycles of [10s at 98ºC, 8min at 66ºC] for the 10kb fragment, or [1min at 95ºC, 18min at 66ºC] for the 23kb fragment and a final extension for 10min at 72ºC. Marker is HyperLadder I (M). The results illustrate that RANGER can be used to amplify products up to 23kb from human genomic DNA, unlike many other competing long-fragment DNA polymerases tested.

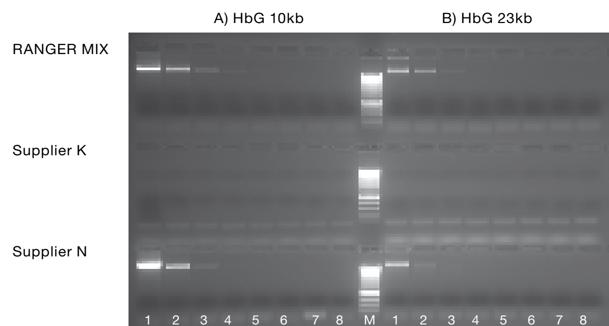


Fig. 2 Efficiency and sensitivity of high-fidelity polymerase mixes
A) A 10kb fragment and B) a 23kb fragment of human β-globin (*HbG*) gene, were amplified using RANGER Mix and the results were compared with amplifications using high-fidelity hot-start DNA mixes from supplier K and supplier N. The process used a serial dilution of human genomic DNA (5ng, 1.6ng, 550pg, 180pg, 60pg, 20pg, 6pg and 0pg human genomic DNA, lanes 1-8 respectively), incubated for 1 min at 95ºC (or according to the manufacturer's protocol) followed by either 30 cycles of [10s at 98ºC, 8min at 66ºC] for the 10kb fragment, or [1min at 95ºC, 18min at 66ºC] for the 23kb fragment and a final extension for 10min at 72ºC. Marker is HyperLadder I (M). The results illustrate that RANGER Mix is more sensitive than other suppliers mixes, particularly with larger fragments.

BIO-X-ACT™ Short DNA Polymerase and Mix

The choice for crude and problematic templates

PRODUCT	PACK SIZE	PRESENTATION	CAT NO.
BIO-X-ACT Short DNA Polymerase	250 Units	1 x 62.50µl	BIO-21064
	500 Units	1 x 125µl	BIO-21065
BIO-X-ACT Short Mix	100 Reactions	2 x 1.25ml	BIO-25025
	500 Reactions	10 x 1.25ml	BIO-25026

FEATURES

- **Ideal for problematic templates**
- **Higher fidelity than *Taq* due to proofreading activity**
- **Perfect for difficult templates that fail with standard *Taq* DNA Polymerases**
- **Amplifies fragments up to 5kb**
- **Available as a ready-to-use 2x reaction mix (BIO-X-ACT Short Mix)**

APPLICATIONS

- **High-fidelity PCR**
- **Suitable for TA cloning**
- **GC-rich templates**
- **Crude sample PCR**

BIO-X-ACT™ Short DNA Polymerase is specifically designed for difficult/problematic PCR applications requiring high processivity and fidelity (fig. 1) that would normally fail with *Taq* DNA polymerase. BIO-X-ACT Short possesses 3'→5' proofreading activity, which in combination with other properties, provides 4-fold higher fidelity than *Taq*.

BIO-X-ACT Short Mix is a complete ready-to-use 2x reaction mix, requiring the simple addition of water, template and primers. In order to achieve optimal reaction conditions, BIO-X-ACT Short Mix contains BIO-X-ACT Short DNA Polymerase, $MgCl_2$, ultra-pure dNTPs manufactured by Bioline plus further additives. The mix has been optimized for a wide variety of templates and reduces the time needed to set up reactions, by reducing the number of pipetting steps that can lead to pipetting errors, thereby minimizing the risk of contamination.

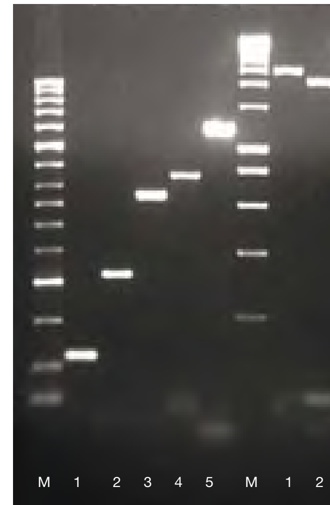
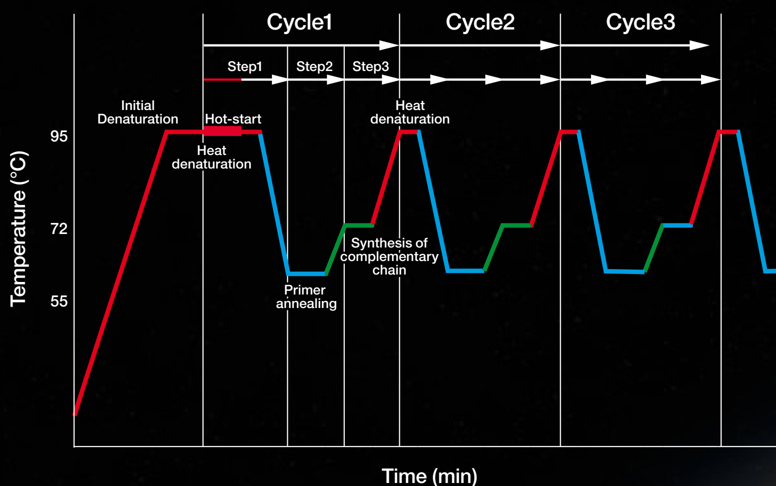


Fig. 1 High specificity on problematic templates
A range of fragments, varying in length and GC content, were amplified with BIO-X-ACT Short Polymerase. These problematic templates originate from a variety of human genes. PCR was performed in 50µl reaction mixtures containing 2.5mM $MgCl_2$. HyperLadder II (M1) and HyperLadder I (M2). The amplification products are: 119bp (43% GC) from human glucocerebrosidase gene (1), 321bp (37% GC) from angiotensin receptor II gene (2), 626bp (56% GC) from rhodopsin gene (3), 762bp (33 % GC) from *β-globin* gene (4), 1200bp (54% GC) from *α-1-antitrypsin* gene (5), 2256bp (52%GC) from the *TP53* gene (6), 2000bp (52% GC) from the angiotensin receptor I gene (7). The results illustrate that BIO-X-ACT Short is robust enough to work with any template.

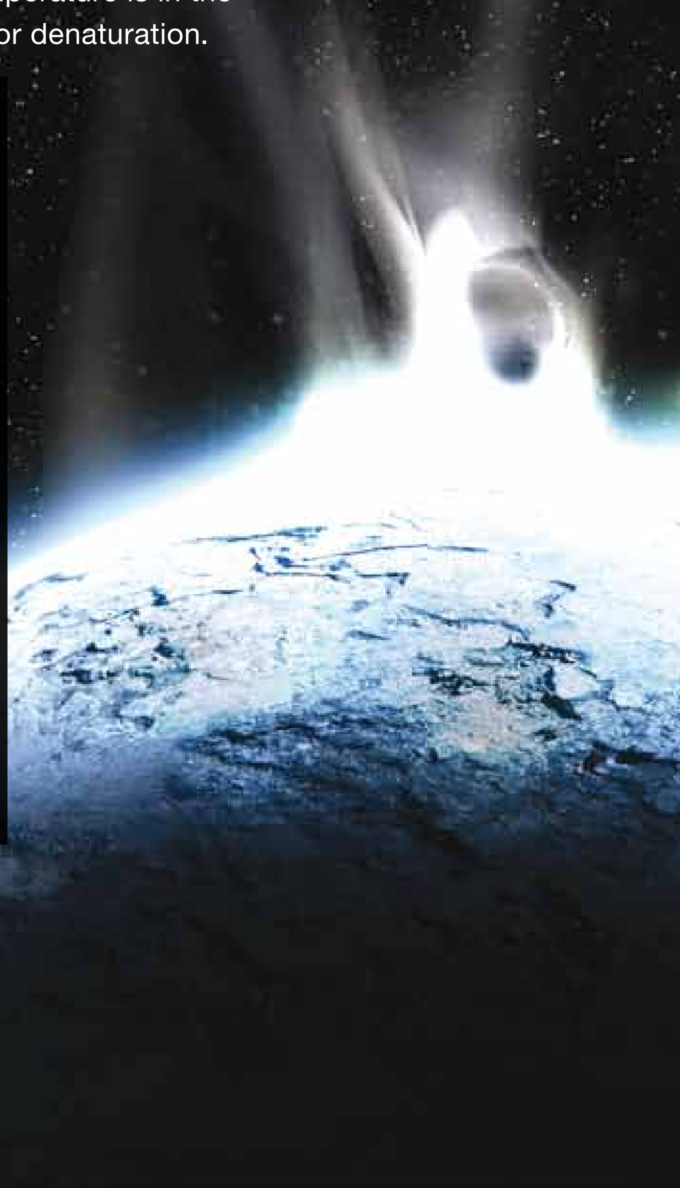
DNA Polymerases for Routine Applications

The PCR process was originally developed to amplify short segments of a longer DNA molecule (1-4). A typical amplification reaction includes target DNA, a thermostable DNA polymerase (originally isolated from the eubacterium *Thermus aquaticus*), two oligonucleotide primers, dNTPs, reaction buffer and magnesium. Each PCR cycle theoretically doubles the amount of targeted sequence (amplicon) in the reaction.

Each cycle of PCR includes steps for template denaturation, primer annealing and primer extension. The initial step denatures the target DNA by heating it to 94°C or higher, to produce single-stranded DNA template for replication. In the next step of a cycle, the temperature is reduced to approximately 40–60°C, allowing the oligonucleotide primers to form stable associations (anneal) with the denatured target DNA and serve as primers to the DNA polymerase. Finally, the synthesis of new DNA begins as the reaction temperature is raised to the optimum for the DNA polymerase. For most thermostable DNA polymerases, this temperature is in the range of 70–74°C. The next cycle begins with a return to 94°C for denaturation.



Each step of the cycle should be optimized for each template and primer pair combination. If the temperature during the annealing and extension steps are similar, these two steps can be combined into a single step in which both primer annealing and extension take place. After 20–40 cycles, the amplified product may be analyzed for size, quantity, sequence, or used in downstream procedures.



MyTaq™ DNA Polymerase and Mix

Best in class

PRODUCT	PACK SIZE	PRESENTATION	CAT NO.
MyTaq DNA Polymerase	500 Units	1 x 100µl	BIO-21105
	2500 Units	2 x 250µl	BIO-21106
	5000 Units	4 x 250µl	BIO-21107
MyTaq Red DNA Polymerase	500 Units	1 x 100µl	BIO-21108
	2500 Units	2 x 250µl	BIO-21109
	5000 Units	4 x 250µl	BIO-21110
MyTaq Mix	200 Reactions	4 x 1.25ml	BIO-25041
	1000 Reactions	20 x 1.25ml	BIO-25042
MyTaq Red Mix	200 Reactions	4 x 1.25ml	BIO-25043
	1000 Reactions	20 x 1.25ml	BIO-25044

FEATURES

- New generation polymerase with superior performance
- Novel buffer system, including dNTPs and $MgCl_2$
- Robust and high yield across a wide range of templates
- Red dye for direct gel loading
- Convenient all-in-one master mix

APPLICATIONS

- High-throughput PCR
- Fast PCR reactions
- Robust amplification of GC-rich sequences
- Site-directed mutagenesis
- TA cloning

The MyTaq™ product range is a new generation of very high performance PCR reagents developed by Bioline. Designed to deliver outstanding results on all templates, including complex genomic DNA templates, MyTaq is based on the latest technology in PCR enzyme preparation, engineered to increase affinity for DNA, resulting in significant improvements to yield, sensitivity and speed. The enzyme is supplied with an industry-leading novel buffer system, specifically formulated and validated for the unique properties of MyTaq, making it the perfect choice for complex templates.

The enzyme is supplied with an industry-leading novel buffer system, a proprietary formulation containing dNTPs, $MgCl_2$ and enhancers at optimal concentrations, removing the need for optimization and providing superior amplification (fig. 1).

MyTaq Mix is a ready-to-use 2x mix for setting up a trouble-free PCR reaction. The advanced formulation of MyTaq Mix allows fast cycling conditions to be used, with greater efficiency, throughput and reproducibility (fig. 2).

MyTaq is also supplied with an inert red dye. Following PCR, samples can be loaded directly onto the agarose gel without the need for a loading buffer, since the mix is of sufficiently high density to sink to the bottom of the well.

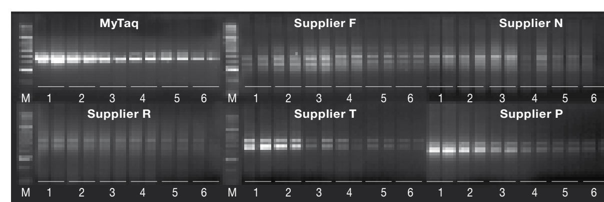


Fig 1. Robust amplification of GC-rich human genomic DNA (61% GC content)
MyTaq was compared with DNA polymerases from other suppliers for the amplification of a 450bp fragment of the human *myc* gene. Decreasing amounts of human genomic DNA were used as a template (1µg, 200ng, 100ng, 50ng, 25ng and 12.5ng; lanes 1-6 respectively) in the PCR. The cycling was performed under the following conditions: 95°C for 5 min, followed by 30 cycles at 95°C for 30s, 60°C for 30s and 72°C for 50s. Marker is HyperLadder I (M) (Cat No. BIO-33025). MyTaq delivers higher yield and sensitivity as compared with all five competing products.

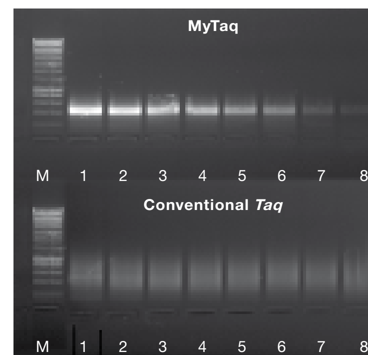


Fig 2. Fast amplification of human genomic DNA (performed in 27.5 minutes)
Comparative amplification of a 450bp fragment of the human *myc* gene (61% GC) was used to compare MyTaq with a conventional *Taq* DNA polymerase. The PCR was performed with both enzymes using decreasing amounts of human genomic DNA as template (200ng, 66ng, 10ng, 3ng, 1ng, 300pg, 100pg and 30pg; lanes 1-8 respectively) and under the following fast cycling conditions: 95°C for 3 min, followed by 30 cycles at 95°C for 15s, 60°C for 15s and 72°C for 15s. Marker is HyperLadder I (M) (Cat No. BIO-33025). In contrast to conventional *Taq*, MyTaq readily copes with faster reaction times, resulting in higher yield without the need for further optimization.

DNA Polymerases for Routine Applications References

1. Mullis, K. *et al. Cold Spring Harbor Syn. Quant. Bio.* **51**, 263-273 (1996).
2. Mullis, K. and Faloona, F. *Methods Enzymology* **155**, 335-350 (1987).
3. Mullis, K. *Scientific American* **262**, 56-61 (1990).
4. Sharkey, D. *et al. Biotechnology* **12**(5), 506-509 (1994).

Mango*Taq*™ DNA Polymerase and MangoMix™

Economic for high-throughput

PRODUCT	PACK SIZE	PRESENTATION	CAT NO.
Mango*Taq* DNA Polymerase	1000 Units	1 x 200µl	BIO-21083
	2000 Units	2 x 200µl	BIO-21082
	5000 Units	5 x 200µl	BIO-21078
MangoMix	250 Reactions	5 x 1.25ml	BIO-25033
	1000 Reactions	20 x 1.25ml	BIO-25034

FEATURES

- **Highly cost effective**
- **Easy visual recognition**
- **Robust performance**
- **Direct loading onto agarose gels**
- **Available as MangoMix, a ready-to-use 2x reaction mix**

APPLICATIONS

- **High-throughput applications**
- **Suited to a wide range of PCR assays**
- **Products suitable for TA cloning**

Mango*Taq*™ DNA Polymerase is a formulation of *Taq* DNA Polymerase which offers consistent results across a wide range of DNA templates (fig. 1). Mango*Taq* DNA Polymerase leaves an 'A' overhang such that the PCR product is suitable for effective integration into TA cloning vectors. For high-throughput applications, Mango*Taq* and the colored reaction buffer make an ideal choice, since this combination enables the user to load directly on a gel (fig. 2), without the need for separate gel-loading buffer. The presence of the dyes has no effect on most routine enzymatic manipulations.

Since the colorless reaction buffer does not contain reference dyes, it is suitable for use when reaction products will be used directly for down-stream processes involving absorbance or fluorescent detection.

MangoMix™ is a complete ready-to-use 2x pre-optimized reaction mix containing Mango*Taq* DNA Polymerase, Mg^{2+}, dNTPs, red and orange reference dyes. MangoMix enables users to perform PCR assays of most common genomic and cDNA templates, simply requiring the addition of water, template and primers to perform the assays. MangoMix dramatically reduces the time required to set up reactions, thereby minimizing the risk of contamination.

Fig. 1 Amplification of different human genes using Mango*Taq* DNA Polymerase and Supplier Q *Taq* DNA Polymerase
The amplification products are as follows: 119bp (43% GC) from human glucocerebrosidase gene (1), 321bp (37% GC) from angiotensin receptor II gene (2), 635bp (56% GC) from rhodopsin gene (3), 762bp (33% GC) from *β-globin* gene (4), 1200bp (54% GC) from *α-1-antitrypsin* gene (5). PCR was performed in 50µl reaction mixtures containing 50ng human genomic DNA and 1.5mM $MgCl_2$. HyperLadder II (M). Mango*Taq* therefore works constantly well over many different templates.

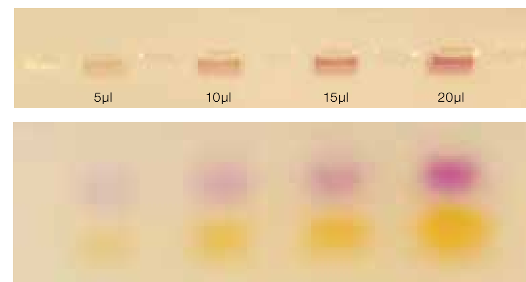


Fig. 2 Mango*Taq* reactions before and after electrophoresis
Volumes below wells indicate loaded volumes of PCR reaction onto a 1% agarose gel using TAE buffer.

BIOTAQ™ DNA Polymerase

The proven standard for PCR

PRODUCT	PACK SIZE	PRESENTATION	CAT NO.
BIOTAQ DNA Polymerase	500 Units	1 x 100µl	BIO-21040
	2500 Units	5 x 100µl	BIO-21060
BIOTAQ PCR Kit	500 Units	1 x 100µl	BIO-21071

FEATURES

- **Consistent delivery of high yield**
- **Suited to a wide range of applications**
- **Processes fragments up to 5kb**
- **Leaves 'A' overhang**

APPLICATIONS

- **Products recommended for TA cloning**
- **Routine PCR applications**

BIOTAQ™ is a highly purified thermostable DNA polymerase offering very high yield over a wide range of PCR templates (fig. 1), and is suitable for most routine assays. BIOTAQ is a robust preparation and consistently delivers high yields with minimal background especially when working with limited amounts of starting material. BIOTAQ leaves an 'A' overhang so that the PCR product is suitable for effective integration into TA cloning vectors. BIOTAQ is supplied with 10x NH_4-based reaction buffer, which provides optimal conditions for most experiments.

The specificity and performance of BIOTAQ can be further improved with the use of 2x PolyMate Additive (Cat No. BIO-37041), which is designed for GC- or AT-rich DNA, "dirty" templates or sequences with a high level of secondary structure.

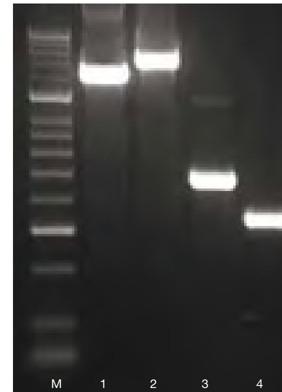


Fig. 1 Amplification of a variety of fragments
BIOTAQ DNA Polymerase was used to amplify a variety of fragments from mouse genomic DNA. Four different genes were amplified with 2.5 Units of BIOTAQ: 1.4kb fragment of *rn18s* gene (lane 1), 1.6Kb fragment of *rn18s* gene (lane 2), 500bp fragment of *Fabpi* gene (lane 3), 350bp fragment of *IL-2* gene (lane 4). PCR was performed in 50µl reaction mixtures containing 1.5mM $MgCl_2$. HyperLadder II (M). BIOTAQ works well over a wide range of PCR templates.

BIOTAQ™ PCR Kit

Ideal for setting up new procedures

FEATURES

- **Designed for easy optimization**
- **High yield over a wide range of PCR templates**
- **Supplied with 2x PolyMate Additive for difficult or "dirty" templates**

APPLICATIONS

- **Products recommended for TA cloning**
- **Routine PCR applications**

The BIOTAQ™ PCR Kit contains all the necessary components to perform PCR assays on a wide range of DNA templates. Optimized levels of ultra-pure dNTPs and BIOTAQ DNA polymerase help ensure sensitive, reproducible results (fig 1).

The BIOTAQ PCR Kit is supplied with 10x NH_4-based reaction buffer, 50mM $MgCl_2$ Solution, 10mM ultra-pure dNTP Mix and 2x PolyMate Additive, ideally suited to dirty/difficult templates with GC or AT-rich DNA, repetitive sequences or sequences with a high level of secondary structure.

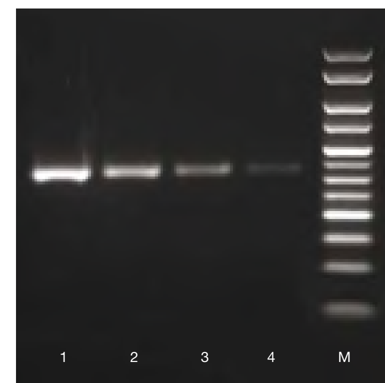


Fig 1. High sensitivity
A 125bp fragment of pGEM plasmid DNA was amplified with 1.0 Unit of BIOTAQ. The fragment was amplified from 15ng plasmid DNA (lane 1) followed by a 10-fold serial dilution of template (lanes 2-4). PCR was performed using 50µl reaction mixtures containing 1.5mM $MgCl_2$. HyperLadder V (M). BIOTAQ PCR kit can therefore provide sensitive PCR of dilute templates.

MyTaq™ One-Step RT-PCR Kit

Sucessful PCR from RNA

PRODUCT	PACK SIZE	CAT NO.
MyTaq One-Step RT-PCR Kit	10 Reactions	BIO-65047
	25 Reactions	BIO-65048
	100 Reactions	BIO-65049

FEATURES

- Extremely sensitive blend of RT and novel hot-start MyTaq
- Highly optimized for detection of low-copy genes
- Overcomes secondary structure in difficult and GC-rich targets
- High-quality, full-length cDNA from as little as 3pg total RNA
- Simple to use all-in-one mix

APPLICATIONS

- Gene-expression analysis
- Transcription analysis
- Gene cloning
- Multiplex RT-PCR

The MyTaq™ One-Step RT-PCR Kit has been formulated for highly reproducible first-strand cDNA synthesis and subsequent PCR in a single tube. A combination of the latest advances in buffer chemistry together with a reverse transcriptase and hot-start DNA polymerase system ensures that MyTaq One-Step RT-PCR Kit produces fast, highly-specific and ultra-sensitive one-step RT-PCR (fig. 1), perfect for all downstream applications.

MyTaq One-Step Kit consists of reverse transcriptase, 2x MyTaq HS Mix and a potent RNase Inhibitor, RiboSafe, that are added together to create a simple to use all-in-one mix.

The kit is ideal for determining the presence or absence of RNA templates and quantifying expression through qualitative, semi-quantitative or quantitative analysis of RNA transcription levels, and the one-step format is also perfect for the synthesis of double-stranded cDNA products for subsequent gene-expression analysis.

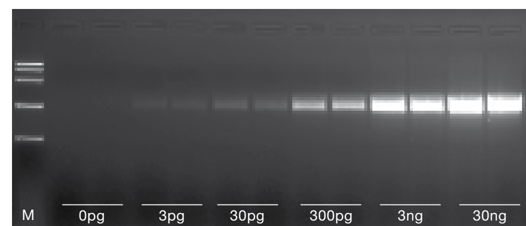


Fig. 1. Sensitivity of MyTaq One-Step RT-PCR Kit
A serial dilution of mouse total RNA (30ng, 3ng, 300pg, 30pg and 3pg respectively) in duplicate was used in a reverse transcription reaction at 45°C for 40min amplified with *RN18S*-1000 primers to produce a 1kb fragment. The cycling was performed under the following conditions: 95°C for 5min and 30 cycles at 95°C for 30s, 58°C for 30s and 72°C for 60s. This was analysed on a 1% agarose gel (BIO-41025). HyperLadder II (M) (BIO-33039). The results illustrate that the MyTaq One-Step RT-PCR Kit is sensitive enough to reverse transcribe and amplify as little as 3pg total RNA.

Ultra-pure dNTP Mixes and Sets

Convenient and pre-optimized

PRODUCT	CONC.	PACK SIZE	CAT NO.
dNTP Mix (dATP, dCTP, dGTP, dTTP)			
dNTP Mix	10mM total	10µmol (1ml)	BIO-39044
	40mM total	20µmol (1 x 500µl)	BIO-39043
	100mM total	50µmol (1 x 500µl)	BIO-39028
	10mM total	100µmol (10 x 1ml)	BIO-39053
	100mM total	200µmol (4 x 500µl)	BIO-39029
	40mM total	1000µmol (5 x 5ml)	BIO-39056
dNTP Set (dATP, dCTP, dGTP, dTTP)			
dNTP Set	100mM total	4 x 25µmol (4 x 250µl)	BIO-39025
	100mM total	4 x 100µmol (4 x 1ml)	BIO-39049
	100mM total	4 x 100µmol (4 x 4 x 250µl)	BIO-39026
	100mM total	4 x 500µmol (4 x 20 x 250µl)	BIO-39027
	100mM total	4 x 1000µmol (4 x 10ml)	BIO-39055

FEATURES

- Ultra-pure >99% triphosphate by HPLC
- Extended shelf-life of 24 months at -20°C
- Free from PCR inhibitors
- DNase, RNase and Nickase free
- Custom, bulk and OEM nucleotides service available

Ultra-pure dNTP sets and mixes are manufactured by Bioline in a purpose-built facility. A ready-to-use molecular grade dNTP Mix containing dATP, dCTP, dGTP and dTTP at pH 7.5 as lithium salts in purified water. The mix is designed to save hands-on time for researchers and minimize the possibility of contamination.

General Considerations and Optimization

Although PCR has become routine in many laboratories, optimal conditions will vary from reaction to reaction and are dependent on the system used. Each parameter can be adjusted individually and some optimization may be required.

Getting started

It is ideal to have a room dedicated for PCR use only. However, this is not possible in many research labs, so try to separate areas used for sample preparation, DNA extraction, amplification and post-PCR analysis and minimize the number of sample manipulations. The PCR bench area used should be decontaminated frequently using a product which removes DNA, as well as cleaned with ethanol (70%) before and after use. Care should also be taken to avoid accidental contamination from the outside environment, this includes use of barrier filter tips and dedicated pipettes (contamination from dirty pipettes is one of the most common causes of experimental failure).

Inclusion of uracil-DNA glycosylase (UNG) and dUTP in all PCRs can help prevent carryover DNA contamination from previous PCRs. However we advise against using this system because it is only effective if all researchers in the laboratory are using the method. If even one researcher is not using this precaution it ceases to be an effective control. Using dUTP has also been shown to reduce reaction efficiency.

Template

The amount of template in the reaction depends mainly on the type of DNA used. For templates with low secondary structural complexity such as plasmid DNA or λ genomic DNA, we recommend using 50pg-10ng DNA per 50µl reaction volume. For complex templates such as human genomic DNA >5kb we recommend a starting amount of 200ng DNA per 50µl reaction, this can be varied between 5ng-500ng. Furthermore, it is important to avoid, where possible, using template re-suspended in EDTA-containing solution (e.g. TE buffer) since EDTA chelates free Mg^{2+}.

Primer preparation

We recommend using primer design software (such as Primer3 or visual OMP); below are the general parameters for most primer software;

1. Generally 20–30 bases is optimal for most PCR regardless of amplicon size.
2. Primers should end (3') in a C or G, or CG or GC. This design increases the efficiency of priming by forming a tight 3' end C/G bond.
3. Design primers with similar Tm values (within 2–3°C of each other). Temperatures between 65–70°C are preferred, as higher annealing temperatures increase reaction specificity.
4. Avoid complementary sequences in the 3'-ends of primers, as primer dimers will be preferentially synthesized.
5. Avoid primer self-complementary sequences (ability to form secondary structures, such as hairpins).
6. Avoid runs of three or more C's or G's at the 3' ends of primers, which may promote mis-priming at C or G-rich sequences.

Primer concentration

Forward and reverse primers are generally used at the final concentration of 0.2-1.0µM each. A final concentration of 0.4µM (i.e. 20pmol of each primer per 50µl reaction volume) should be sufficient for most PCR. Too high primer concentration can reduce the specificity of the priming, increasing the chance of non-specific amplification.

Primer annealing

The annealing temperature depends upon the melt-temperature (Tm) of the primers, usually 2-5°C below the lower Tm of the pair. We recommend starting with a 55°C annealing temperature and if necessary to run a temperature gradient from 50-65°C to determine the optimal annealing temperature.

A good estimation is Tm = 2°C x (number of A+T) + 4°C x (number of G+C)

Polymerase

Bioline's product offering for PCR includes a variety of DNA polymerases and mixes providing a suitable enzyme for all purposes, from routine PCR to the most demanding high-fidelity applications. The selection table at the start of this guide will assist you to choose the most suitable polymerase for your application.

We suggest starting with the lowest concentration and not to exceed the highest (Table 1), since excess enzyme may facilitate non-specific amplification which can result in a diffuse smear of bands. In contrast, insufficient enzyme lowers the efficiency of amplification which may result in low or no product yields.

The extension step should be performed at 72°C. The extension time depends on the length and type of the product to be amplified, in general the shorter times below for amplicons <5kb (Table 1) (see individual product sheets for more details).

ENZYME	RXN SIZE	UNITS	TIME/KB
MyTaq	50 µL	1.0-5.0 Units	10 s
MyFi	25 µL	2.0 Units	15-45 s
RANGER	50 µL	4.0 Units	45-60 s
BIOTAQ	50 µL	2.5-5.0 Units	15-30 s
Mango*Taq*	50 µL	2.5-5.0 Units	15-30 s
MyTaq HS	50 µL	1.0-5.0 Units	10 s
IMMOLASE	50 µL	1.0-5.0 Units	15-30 s
VELOCITY	50 µL	0.25-2.0 Units	15 s
ACCUZYME	50 µL	0.25-2.0 Units	1.5-2.0 min
BIO-X-ACT	50 µL	4.0 Units	1.0 min

Table 1. Concentrations and extension times needed for DNA polymerases

Cycle numbers

For most PCR reactions the optimum cycle number is 25–30 cycles. The exact number should be determined by considering the quantity or complexity of template DNA and the amplicon length. Insufficient cycles may result in low product yield, whereas excess cycles may encourage amplification of secondary products or contaminants, resulting in spurious bands or a smear upon electrophoresis.

Magnesium concentration

Magnesium concentration is a critical factor in a PCR reaction, affecting the fidelity of the DNA polymerase. Optimal magnesium concentration is affected by dNTP and template concentrations, template-primer combinations, and chelating agents such as EDTA which could be carried over with template DNA.

Magnesium affects the annealing of the primer to the template DNA by stabilizing the base pairing interactions. An excess of Mg^{2+} in the reaction will promote secondary structure elements and increase non-specific primer binding leading to non-specific products. Alternatively, too low a concentration will decrease the reaction yield.

Generally a 1:2 ratio (dNTP:Mg^{2+}) is optimal, we therefore recommend a final Mg^{2+} concentration of 2mM, but some optimization may be necessary, especially if using dNTP concentration higher than the recommended one.

dNTPs purity and concentration

It is important that the dNTPs are pure and stable, for optimal results we recommend using Bioline ultra-pure dNTPs in a balanced mix. Optimal dNTP concentration in most PCR reactions is 250µM or less for each dNTP. At lower than optimum concentrations, amplification yield may be poor. At a higher than optimal concentration, the degree of nucleotide misincorporation will increase.

Buffers and enhancing reagents

The default buffer supplied with the polymerases has been designed to give high yield for the majority of standard templates.

Several additives are commonly used however to enhance PCR performance for difficult templates such as human genomic DNA or those possessing high-GC-content or complex structural organisation. They include PolyMate Additive (BIO-37041), Hi-Spec Additive (BIO-37032) and dimethyl sulfoxide (DMSO), they all act by “melting” secondary structures and decreasing non-specific products, thus improving amplification efficiency as well as specificity. The usefulness of these adjuvants must be tested for each experiment to determine their value.

DNA amplification

DNA amplification consists of two stages, stage 1 is a denaturation (polymerase activation) step and stage 2 is a 3-step cycle (consisting of denaturation, annealing and extension).

Stage 1 – Denaturation (polymerase activation)
During the initial denaturation step, the double helix is melted into single-stranded DNA molecules. Temperatures above 95°C are not recommended for *Taq* based PCR denaturation due to the thermal stability of *Taq* DNA polymerase (*Taq* half-life at 95°C = 35 min vs. 97.5°C = 7 min). Proofreading polymerases such as Bioline’s VELOCITY DNA polymerase however can use a 98°C denaturation temperature.

Stage 1 is extended for hot-start polymerases for 1 to 10 minutes, depending on the polymerase. The hot-start prevents the polymerase from working at lower temperatures, providing improved specificity and eliminating the presence of non-specifics, such as primer-dimers and mis-primed products.

Stage 2 – Amplification of target DNA sequence: 3 step cycling (denaturation, annealing and extension).

Denaturation: This functions to denature newly synthesized PCR products (i.e., products from the previous amplification cycle).

Annealing: Following denaturation, the temperature is lowered to Tm determined for primers used (normally around 55°C) to facilitate binding of the primers to complementary sequences of the target amplicon. Initial primer elongation then allows for the temperature to be increased to 72°C.

Extension: During the extension step the temperature is subsequently raised to 72°C, being the optimal temperature for nucleotide addition. The time needed will vary depending on the polymerase used (see above). Nucleotides continue to be added to the 3' end of the nascent strand until the temperature is raised again to 95°C (denaturation step), beginning the next round of cycling. This 3-step cycle is generally repeated ~30 times, and ultimately results in the production of ~1 billion copies of the target molecule.

Troubleshooting

Problem	Possible Cause	Recommendation
No PCR product	Missing component	- Check mix set-up and volumes used
	Defective component	- Check the aspect and the concentrations of all components as well as the storage conditions. If necessary test each component individually in control reactions
	Enzyme concentration too low	- Increase enzyme quantity (see Table 1)
	Cycling conditions not optimal	- Decrease the annealing temperature - Run a temperature gradient to determine the optimal annealing temperature - Increase the extension time, especially if amplifying a long target - Increase the number of cycles
	Not enough Mg^{2+}	- Increase the $MgCl_2$ concentration in 0.5mM increments
	Difficult template	- Increase the denaturation time - Try temperature gradient to find the optimal annealing temperature - Try addition of enhancement reagent, or try polymerase that is better suited to difficult templates (such as VELOCITY)
	Primers not matched	- Redesign primers using primer design software (e.g. Primer3)
	Template quality poor	- Check quality and concentration of template - Amplify template with an established primer pair or in an established PCR system - Check purity of template on a gel. If template is impure or degraded, repeat purification - Try $MgCl_2$ concentrations between 1.5 and 5mM - Try additive to increase reaction efficiency - Try a serial dilution to see if inhibitors are present - Try primers that amplify a smaller target
Smearing or non- specific products	Excessive cycling	- Decrease the number of cycles
	Extension time too long	- Decrease the extension time
	Annealing temperature too low	- Increase the annealing temperature - Use additive such as PolyMate Additive, Hi-Spec Additive or DMSO (do not use with MyTaq, MyFi or RANGER)
	Non-specific annealing of primers due to room temperature set-up	- Change to a hot-start polymerase
	Too much enzyme	- Decrease enzyme concentration
	Primer concentration too high	- Decrease primer concentration
	Primer design not specific enough	- Design primers with high specificity to target DNA - Increase length of primers
	Contamination	- Replace each components in order to find the possible source of contamination - Set-up the PCR reaction and analyze the PCR product in separated areas.
	Difficult template (e.g. GC rich template)	- Try additive that will reduce melting temperature such as DMSO (do not use with MyTaq, MyFi or RANGER) - Try a more robust DNA polymerase, designed for difficult templates
	Secondary amplification product	- Check reagent concentration and cycle conditions - Decrease number of cycles - Verify (and possibly decrease) template concentration - Use less polymerase in reaction

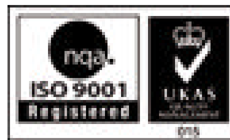
Bulk, Custom and OEM Services

Creative Solutions to suit your needs

When your requirements are beyond the scope of our standard product range you can take advantage of our bulk, custom and OEM service. As a primary manufacturer of enzymes, Bioline can accommodate requests from micro-liter to multi-liter quantities. We can manufacture special batches with unique formulations, blends and mixes to your specific requirements. Private labeling and packaging arrangements are also possible, that include:

- Custom product configurations
- Custom bulk packaging requirements
- Private label OEM manufacturing
- Validation support information
- Scheduled delivery to fit your needs
- Fast-track technical and scientific support
- Mutually agreed confidentiality

Our ISO 9001:2008 certification is a key element in our business and provides you with the confidence and trust you need. Certification and its enforcement mean consistency in our processes, reproducible quality in our products, rigorous QA/QC, retention of samples if needed and complete product documentation and traceability.



Visit www.bioline.com/custom for more information.

SensiFAST™ Real-Time PCR Kits

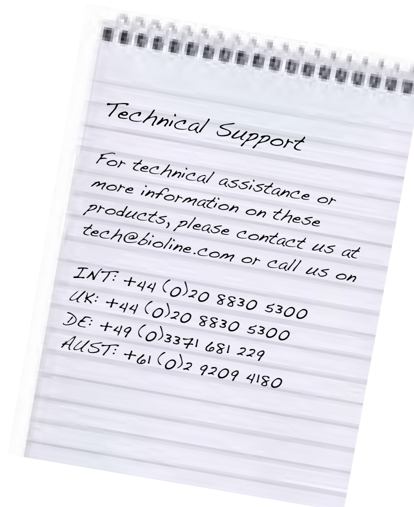
Bioline offers SensiFAST™, a comprehensive range of highly optimized products designed to deliver outstanding results for real-time experiments with both DNA and RNA templates, providing reliable and highly reproducible data on all commonly used real-time PCR instruments. For further information on real-time PCR, please go to www.bioline.com/sensifast

Ordering Information

PRODUCT	PACK SIZE	PRESENTATION	CAT NO.
Hot-Start DNA Polymerases			
MyTaq HS DNA Polymerase	250 Units	1 x 50µl	BIO-21111
	1000 Units	1 x 200µl	BIO-21112
	2500 Units	2 x 250µl	BIO-21113
MyTaq HS Red DNA Polymerase	250 Units	1 x 50µl	BIO-21114
	1000 Units	1 x 200µl	BIO-21115
	2500 Units	2 x 250µl	BIO-21116
MyTaq HS Mix	200 Reactions	4 x 1.25ml	BIO-25045
	500 Reactions	10 x 1.25ml	BIO-25046
MyTaq HS Red Mix	200 Reactions	4 x 1.25ml	BIO-25047
	500 Reactions	10 x 1.25ml	BIO-25048
IMMOLASE DNA Polymerase	250 Units	1 x 50µl	BIO-21046
	500 Units	1 x 100µl	BIO-21047
	5000 Units	10 x 100µl	BIO-21048
MyFi DNA Polymerase	250 Units	1 x 125µl	BIO-21117
	500 Units	1 x 250µl	BIO-21118
	2500 Units	2 x 625µl	BIO-21119
MyFi Mix	100 Reactions	2 x 1.25ml	BIO-25049
	500 Reactions	10 x 1.25ml	BIO-25050
High-Fidelity DNA Polymerases			
VELOCITY DNA Polymerase	250 Units	1 x 125µl	BIO-21098
	500 Units	1 x 250µl	BIO-21099
VELOCITY PCR Kit	250 Units	20 Reactions	BIO-21104
PCR Tailing Mix	50 Reactions	1 x 250µl	BIO-21103
ACCUZYME DNA Polymerase	250 Units	1 x 100µl	BIO-21051
	500 Units	1 x 200µl	BIO-21052
ACCUZYME Mix	100 Reactions	2 x 1.25ml	BIO-25027
	500 Reactions	10 x 1.25ml	BIO-25028
DNA Polymerases for Specialized Applications			
RANGER DNA Polymerase	250 Units	1 x 62.50µl	BIO-21121
	500 Units	1 x 125µl	BIO-21122
	2500 Units	2 x 312.50µl	BIO-21123
RANGER Mix	100 Reactions	2 x 1.25ml	BIO-25051
	500 Reactions	10 x 1.25ml	BIO-25052
BIO-X-ACT Short DNA Polymerase	250 Units	1 x 62.50µl	BIO-21064
	500 Units	1 x 125µl	BIO-21065
BIO-X-ACT Short Mix	100 Reactions	2 x 1.25ml	BIO-25025
	500 Reactions	10 x 1.25ml	BIO-25026
DNA Polymerases for Routine Applications			
MyTaq DNA Polymerase	500 Units	1 x 100µl	BIO-21105
	2500 Units	2 x 250µl	BIO-21106
	5000 Units	4 x 250µl	BIO-21107
MyTaq Red DNA Polymerase	500 Units	1 x 100µl	BIO-21108
	2500 Units	2 x 250µl	BIO-21109
	5000 Units	4 x 250µl	BIO-21110
MyTaq Mix	200 Reactions	4 x 1.25ml	BIO-25041
	1000 Reactions	20 x 1.25ml	BIO-25042
MyTaq Red Mix	200 Reactions	4 x 1.25ml	BIO-25043
	1000 Reactions	20 x 1.25ml	BIO-25044
BIOTAQ DNA Polymerase	500 Units	1 x 100µl	BIO-21040
	2500 Units	5 x 100µl	BIO-21060
BIOTAQ PCR Kit	500 Units	1 x 100µl	BIO-21071

PRODUCT	PACK SIZE	PRESENTATION	CAT NO.
Mango*Taq* DNA Polymerase	1000 Units	1 x 200µl	BIO-21083
Mango*Taq* DNA Polymerase	2000 Units	2 x 200µl	BIO-21082
	5000 Units	5 x 200µl	BIO-21078
MangoMix	250 Reactions	5 x 1.25ml	BIO-25033
	1000 Reactions	20 x 1.25ml	BIO-25034
Nucleotides			
dNTP Mix (dATP, dCTP, dGTP, dTTP)			
dNTP Mix	10mM total	10µmol (1ml)	BIO-39044
	40mM total	20µmol (1 x 500µl)	BIO-39043
	100mM total	50µmol (1 x 500µl)	BIO-39028
	10mM total	100µmol (10 x 1ml)	BIO-39053
	100mM total	200µmol (4 x 500µl)	BIO-39029
	40mM total	1000µmol (5 x 5ml)	BIO-39056
dNTP Set (dATP, dCTP, dGTP, dTTP)			
dNTP Set	100mM total	4 x 25µmol (4 x 250µl)	BIO-39025
	100mM total	4 x 100µmol (4 x 1ml)	BIO-39049
	100mM total	4 x 100µmol (4 x 4 x 250µl)	BIO-39026
	100mM total	4 x 500µmol (4 x 20 x 250µl)	BIO-39027
	100mM total	4 x 1000µmol (4 x 10ml)	BIO-39055
DNA Polymerases for Gene Expression			
MyTaq One-Step RT-PCR Kit	10 Reactions	-	BIO-65047
	25 Reactions	-	BIO-65048
	100 Reactions	-	BIO-65049

PRODUCT	PACK SIZE	CONC.	CAT NO.
PCR Buffers			
10x NH_4 Buffer	3 x 1.2ml	10x	BIO-37025
Mango*Taq* Buffer Colorless	10 x 1ml	5x	BIO-37101
Mango*Taq* Buffer Colored	10 x 1ml	5x	BIO-37102
MyTaq Reaction Buffer Colorless	4 x 1ml	5x	BIO-37111
MyTaq Reaction Buffer Red	4 x 1ml	5x	BIO-37112
$MgCl_2$ Solution (50mM)	3 x 1.2ml	NA	BIO-37026



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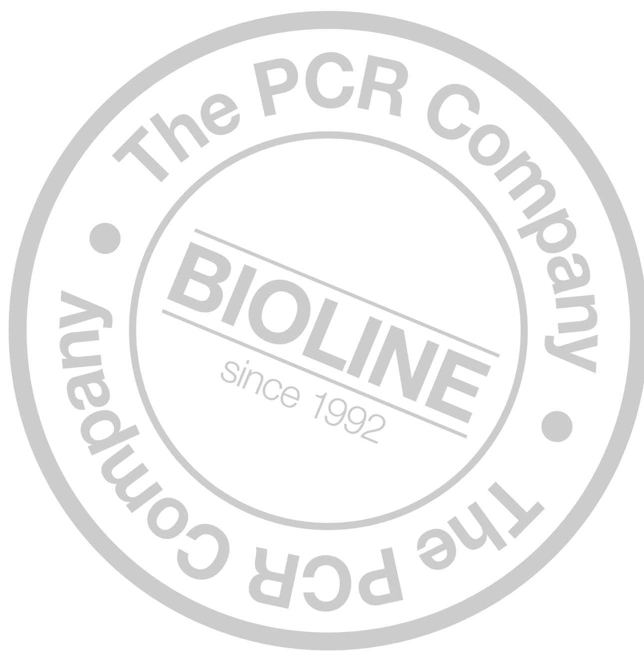

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