A New Generation of End-Point PCR

PCR Enzyme Guide

A New Generation of End-Point PCR
Bioline the PCR Company

For 22 years, Bioline has been developing and manufacturing a complete portfolio of high performance PCR enzymes, for a wide range of applications. During this time, we have analyzed a large number of enzymes, buffer systems and components in order to fully understand the reaction kinetics and processes that lead to high efficiency PCR. Our research in this area has led to the introduction of the proven MyTaq™, MyFi™ and RANGER product ranges, the new generation of Bioline DNA polymerases for reliable and reproducible high-sensitivity, high-efficiency PCR.

Superior handling

Not only have we developed performance leading polymerases, but we have also improved the handling and ease-of-use in PCR experiments. Many of our most popular PCR enzymes are available in practical, ready to use 2x mastermixes: These mixes have been pre-optimized to deliver the best results. Simply add template and primers to achieve high-sensitivity and high-efficiency results for your next publication or report. Many polymerases and mixes have the added advantage of the inclusion of an inert red dye to improve visualization and enable direct gel loading.

Bioline DNA polymerases

Each DNA polymerase has characteristics matched to specific PCR applications. In order to obtain the best results from a particular experiment, it is important to select the polymerase best suited to the application. This guide is intended to assist you in the selection of the most appropriate DNA polymerase for your assay.

Our products speak for themselves

We invite you to visit Bioline Scholar, a compilation of publications citing our reagents and The PCR Challenge, comparative PCR data provided by our customers globally. As our core expertise and focus is the field of PCR, we are proud to be called The PCR Company.
Choosing the Right DNA Polymerase

Successful PCR depends on two crucial components, an optimized reaction buffer and a high-quality, thermostable DNA polymerase (such as Taq DNA polymerase).

Four basic properties of DNA polymerases define the best enzyme for your particular research needs: thermal stability, extension rate, fidelity (the ability to replace incorrectly incorporated nucleotides) and processivity (the probability a polymerase will detach from DNA during extension). Different configurations of these four variables have produced different classes of DNA polymerase, namely:

### Standard DNA polymerases
Suitable for routine PCR, such as detection of amplified product and estimation of product size, producing a single-based ‘A’ overhang, enabling direct insertion into T/A cloning vectors.

### Hot-start (HS) polymerases
Used to suppress nonspecific product amplification during setup and to increase yield of the desired product. Hot-start is useful when DNA template amounts are low; DNA templates are highly complex or several pairs of primers are used, as in multiplex PCR.

### High-fidelity polymerases
These remove erroneous bases incorporated in the growing DNA strand, increasing the accuracy of DNA synthesis from template DNA. For cloning and expression of amplified product, mutagenesis studies and related applications, proofreading enzymes should be used.

### Polymerases for amplification of long amplicons
Amplification of long amplicons combines the processivity of standard DNA polymerases with the accuracy of a proofreading polymerase. This is achieved by blending two polymerases with an optimized buffer, to give amplicons as long as 25kb from genomic DNA.

### Applications

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### Properties

- **Hot-Start**
  - High-fidelity DNA polymerase is used to suppress non-specific product amplification and primer-dimer formation during set-up, to increase the yield of the desired product. Hot-start PCR is very useful when the amount of DNA template is very low, when the DNA template is highly complex and in multiplex PCR.

- **High-Fidelity**
  - Fidelity refers to the frequency of insertion of an incorrect nucleotide by a polymerase. High-fidelity DNA polymerases minimize the introduction of amplification errors and are used for product that will be cloned, sequenced and expressed. They save time and effort by eliminating the need for downstream screening in order to obtain error-free constructs.

- **High Specificity**
  - The specificity of a PCR is how likely it is to produce one and only one amplification product that is the intended target sequence. This is not only related to the hot-start, but also the buffer composition and how well it has been optimized to the DNA polymerase.

- **Standard PCR**
  - A standard DNA polymerase is used for general purpose PCR, such as detection of the amplified product and the estimation of product size etc. They produce fragments with ‘A’ overhang at 3’-end, allowing direct cloning into T/A cloning vectors.

- **Long PCR**
  - Long PCR refers to the amplification of DNA lengths that cannot typically be amplified using routine polymerases and reagents.

- **Pre Mix**
  - Newer DNA polymerases have extension rates of less than 1kb/30 seconds giving total cycling times of less than 30 minutes. Fast PCR is therefore ideal for high throughput.

- **Multiplex PCR**
  - Multiplex PCR consists of multiple primer sets within a single PCR mixture to produce amplicons of varying sizes that are specific to different DNA sequences. Multiplex PCR is ideal for screening assays where the same amplicons are compared in multiple samples.

- **Genotyping PCR**
  - Genotyping PCR is the process of determining differences in the genetic make-up of individuals, by amplifying and comparing specific regions of the genomes.

- **Low-Copy PCR**
  - Low-copy templates require highly-sensitive and specific DNA polymerases to reduce the risk of non-specific bands causing smearing or false positive results.

- **Direct Gel Loading**
  - An inert red dye is available in a number of our polymerase buffers. Following PCR, samples can be loaded directly onto the agarose gel without the need for a loading buffer, improving ease-of-use.

For more information please visit [www.bioline.com/polymersases](http://www.bioline.com/polymersases)
MyTaq™ HS DNA Polymerase and Mix

MyTaq™ HS is a very high-performance, antibody-mediated hot-start DNA polymerase, designed for fast, highly-specific PCR.

**APPLICATIONS**
- Fast PCR reactions
- Colony PCR
- Genotyping
- Multiplexing
- Low-copy PCR assays
- Specific amplification of difficult templates (GC-rich)
- Assays with prolonged reaction set-up

**FEATURES**
- Universal DNA polymerase - recommended for most applications
- Complete PCR reaction in less than 30 minutes
- Antibody-based hot-start polymerase
- Highest specificity and superior performance
- Highly optimized buffer system, including ultra-pure dNTPs and MgCl₂
- Available with red dye for direct gel loading
- All-in-one mastermix formulations enhance reproducibility

**RECOMMENDED FOR**
- Genotyping
- High-throughput PCR
- Fast PCR reactions
- GC-rich amplification

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**MyTaq™ DNA Polymerase and Mix**

MyTaq™ is a fast, very high-performance DNA polymerase designed to deliver outstanding results on a wide-range of templates.

**APPLICATIONS**
- Genotyping
- High-throughput PCR
- Fast PCR reactions
- GC-rich amplification

**FEATURES**
- New generation polymerase with superior performance
- Novel buffer system, including dNTPs and MgCl₂
- Available with red dye for direct gel loading
- Convenient all-in-one mastermix options available

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**Fig. 1** Reduced time of MyTaq HS in Colony PCR

A plate of 96-well plates was set up with A, B, C and D inserts. A 340bp, 450bp, 525bp and 530bp fragment (A-D respectively) was amplified with MyTaq HS PCR reactions. The results illustrated that MyTaq HS is robust enough to amplify repetitively and successfully the targeted DNA directly from colonies. Hyperladder™ 1kb (M).

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**Fig. 2** Fast amplification (23.5 sec incubation) was carried out on a range of human genomic genes

1. 200bp 2. 300bp 3. 400bp 4. 500bp 5. 600bp 6. 700bp. MyTaq HS PCR reactions were performed with competing products from other suppliers, including an antibody-based polymerase (MyTaq). Hyperladder™ 1kb (M).

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**Fig. 3** Fast amplification of human genomic DNA (37°C, GC-content)

MyTaq was compared with other DNA polymerase forms using DNA from a 37°C, GC-content. MyTaq HS PCR reactions were performed with competing products from other suppliers. Hyperladder™ 1kb (M).

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**Fig. 4** Fast amplification of human genomic DNA (performed in 37°C, GC-content)

MyTaq was compared with other DNA polymerase forms using DNA from a 37°C, GC-content. MyTaq HS PCR reactions were performed with competing products from other suppliers. Hyperladder™ 1kb (M).

---

**Fig. 5** Fast amplification of human genomic DNA (performed in 37°C, GC-content)

MyTaq was compared with other DNA polymerase forms using DNA from a 37°C, GC-content. MyTaq HS PCR reactions were performed with competing products from other suppliers. Hyperladder™ 1kb (M).
MyFi™ DNA Polymerase and Mix

MyFi™ is a novel, antibody-mediated DNA polymerase complex with enhanced sensitivity and fidelity, ideally suited to problematic DNA templates.

**RECOMMENDED FOR**
- New generation DNA polymerase complex provides robust PCR with 3.5-fold higher fidelity than wild-type Taq polymerase
- Antibody-based hot-start polymerase enhances specificity and allows room-temperature reaction set-up
- Efficiently amplifies complex mammalian DNA fragments up to 10kb
- Novel 5x buffer system ensures reliability, reproducibility and convenience
- Also available as a ready-to-use, all-in-one 2x mastermix

**APPLICATIONS**
- TA Cloning
- Long PCR
- Low-copy PCR assays
- PCR of difficult DNA templates (problematic GC/AT-rich genomic DNA)
- High-fidelity PCR assays (library amplification)

**FEATURES**
- High-fidelity PCR assays (library amplification)
- Low-copy PCR assays
- PCR of difficult DNA templates (problematic GC/AT-rich genomic DNA)
- TA Cloning
- Long PCR

**RECOMMENDED FOR**
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- Low-copy PCR assays
- PCR of difficult DNA templates (problematic GC/AT-rich genomic DNA)
- TA Cloning
- Long PCR

**PRODUCT**

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>PACK SIZE</th>
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<td>MyFi Mix</td>
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<td>MyFi Mix</td>
<td>500 Reactions</td>
<td>10 x 1.25ml</td>
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**APPLICATIONS**

- High-fidelity PCR assays (library amplification)
- Low-copy PCR assays
- PCR of difficult DNA templates (problematic GC/AT-rich genomic DNA)
- TA Cloning
- Long PCR

**FEATURES**

- High-fidelity PCR assays (library amplification)
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- High-fidelity PCR assays (library amplification)
- Low-copy PCR assays
- PCR of difficult DNA templates (problematic GC/AT-rich genomic DNA)
- TA Cloning
- Long PCR

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RANGER DNA Polymerase and Mix

RANGER DNA Polymerase 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.

**RECOMMENDED FOR**
- Fast antibody-based hot-start
- Unique buffer system, including ultra-pure dNTPs and MgCl₂
- Higher-fidelity than Taq
- Available as a convenient all-in-one mastermix for ease of set-up

**APPLICATIONS**

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

**FEATURES**

- Fast antibody-based hot-start
- Unique buffer system, including ultra-pure dNTPs and MgCl₂
- Higher-fidelity than Taq
- Available as a convenient all-in-one mastermix for ease of set-up

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- Validated for human genomic DNA up to 25kb
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<td>RANGER DNA Polymerase</td>
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<td>BIO-21121</td>
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<tr>
<td>RANGER DNA Polymerase</td>
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<td>2 x 125μl</td>
<td>BIO-21122</td>
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<td>RANGER Mix</td>
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<td>2 x 50μl</td>
<td>BIO-21123</td>
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<td>RANGER Mix</td>
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<td>BIO-21124</td>
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<td>RANGER Mix</td>
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<td>BIO-21125</td>
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**APPLICATIONS**

- Fast antibody-based hot-start
- Unique buffer system, including ultra-pure dNTPs and MgCl₂
- Higher-fidelity than Taq
- Available as a convenient all-in-one mastermix for ease of set-up

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www.bioline.com | The PCR Company

For more information please visit www.bioline.com/polymerases
VELOCITY DNA Polymerase

VELOCITY DNA Polymerase is an ultra-fast thermostable enzyme possessing 5'→3' proofreading exonuclease activity, which makes it ideal for high-fidelity PCR.

**RECOMMENDED FOR**

- HF
- F
- LC

**FEATURES**

- High-fidelity DNA polymerase
- High-processivity
- Fast amplification
- Ideal for a wide range of templates including long templates greater than 5kb

**APPLICATIONS**

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

VELOCITY delivers outstanding PCR yield with exceptional fidelity error rates of less than 4.4 x 10^{-7} bases, even from low template concentrations. High-processivity and extension rates result in shorter extension times in assays including those that are complex or contain impurities (Fig. 1).

ACCUZYME™ DNA Polymerase and Mix

ACCUZYME™ is a thermostable enzyme possessing 5'→3' DNA polymerase and 3'→5' proofreading exonuclease activities, offering high-fidelity.

**RECOMMENDED FOR**

- HF
- LC

**FEATURES**

- High-fidelity DNA polymerase
- Amplifies fragments up to 5kb
- Available as a convenient pre-mix (ACCUZYME Mix)

**APPLICATIONS**

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

ACCUZYME™ is a high-fidelity (proofreading) polymerase that produces blunt-ended amplicons up to 5kb. ACCUZYME possesses very high PCR sensitivity and is ideally suited to low-copy target amplifications (Fig. 1). ACCUZYME Mix dramatically reduces the time needed to set up reactions, thereby minimizing the risk of contamination.

MyTaq™ Extract-PCR Kit

The MyTaq™ Extract-PCR Kit offers a quick and easy alternative for the extraction and amplification of DNA from a variety of tissue types.

**RECOMMENDED FOR**

- HF
- F
- M
- HS
- D

**FEATURES**

- Rapid extraction protocol: High yield PCR-ready DNA in about 15 minutes
- Replaces complicated DNA extraction procedures
- Perfect for high-throughput genotyping from mammalian tissues
- Convenient single-tube reaction minimizes contamination
- MyTaq HS Red Mix for fast and highly-specific amplification and direct gel loading

**APPLICATIONS**

- Ideal for high-throughput genotyping from mammalian tissues
- Detection of transgenes
- Knockout analysis

Many DNA extraction methods can be laborious and time consuming, involving the use of hazardous chemicals. MyTaq Extract-PCR Kit offers a rapid and easy alternative for the extraction and amplification of DNA from a variety of tissue types. MyTaq Extract-PCR Kit is particularly suited to solid tissues such as mouse tail and ear.

The extracted DNA is amplified in a proprietary buffer system using MyTaq HS Red Mix, to give high sensitivity and very high yields, as well as allowing fast cycling times for direct gel-loading for high throughput assays.

When used with the same starting material, MyTaq Extract-PCR Kit gives a better yield and is more sensitive, compared to other suppliers of similar kits. The kit offers a convenient alternative for the extraction of DNA for applications such as mouse genotyping and sequencing (Fig. 1).
**MyTaq™ Blood-PCR Kit**

MyTaq™ Blood-PCR Kit offers fast, highly-specific, direct PCR from whole blood samples.

**PRODUCT**

- MyTaq Blood-PCR Kit

**PACK SIZE**

- 100 Reactions
- 250 Reactions

**CAT NO.**

- BIO-25053
- BIO-25054

**RECOMMENDED FOR**

- F
- M
- G

**FEATURES**

- Extraction-free, eliminates complex DNA extraction protocols
- Novel buffer system designed to overcome blood inhibition
- MyTaq™ HS Mix for fast and highly-specific amplification
- Ideal for multiplexing, GC-rich templates and longer amplicons

**APPLICATIONS**

- Human and animal blood extraction and amplification
- Blood preserved with heparin, citrate or EDTA

MyTaq Blood-PCR Kit is highly optimized for use with whole blood collected with various anticoagulants (EDTA, citrate, heparin) from both human and non-human origins.

MyTaq Blood-PCR Kit has been specifically developed to overcome PCR inhibitors typically present in blood samples to give significantly increased sensitivity and PCR success rates (Fig. 1).

The advanced formulation of MyTaq Blood-PCR Kit allows the use of fast cycling conditions without compromising PCR specificity and yield. The speed and high specificity of MyTaq Blood-PCR Kit also makes it highly suitable for multiplex PCR applications.

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**MyTaq™ One-Step RT-PCR Kit**

The MyTaq™ One-Step RT-PCR Kit has been formulated for highly reproducible first-strand cDNA synthesis and subsequent PCR in a single tube.

**PRODUCT**

- MyTaq One-Step RT-PCR Kit

**PACK SIZE**

- 25 Reactions
- 100 Reactions

**CAT NO.**

- BIO-65049

**RECOMMENDED FOR**

- HS
- F
- LC
- G

**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
- Available as a simple to use all-in-one mix

**APPLICATIONS**

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

MyTaq One-Step RT-PCR Kit uses the latest advances in buffer chemistry combined with a novel reverse transcriptase and hot-start DNA polymerase. This 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 RT-PCR Kit consists of reverse transcriptase, 2x MyTaq HS Mix and a potent RNase Inhibitor, RiboSafe, that collectively 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. The one-step format is also perfect for the synthesis of double-stranded cDNA products for subsequent gene-expression analysis.

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**MyTaq™ Blood-PCR Kit**

**PRODUCT**

- MyTaq Blood-PCR Kit

**PACK SIZE**

- 100 Reactions
- 250 Reactions

**CAT NO.**

- BIO-25053
- BIO-25054

**RECOMMENDED FOR**

- HS
- F
- M
- G

**FEATURES**

- Extraction-free, eliminates complex DNA extraction protocols
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EPIK™ Amplification Kit

EPIK™ Amplification Kit is a ready-to-use, PCR mix engineered to overcome the challenges associated with bisulfite-modified DNA.

**FEATURES**
- Outstanding reliability, engineered for best-in-class epigenetic analysis time after time
- Dedicated EPiKORE™ buffer system, optimized for amplification of bisulfite-modified DNA
- Engineered to amplify fragments with low, medium and high GC content without bias
- Hot-start system powered by MyTaq™ HS for high specificity
- Enhanced polymerization for longer amplicons
- Higher yield even with low DNA template

**APPLICATIONS**
- Bisulfite-modified, uracil containing DNA (up to 1.5kb)
- Difficult DNA templates e.g. GC-rich
- Bisulfite-restriction PCR e.g. COBRA
- Bisulfite-sequencing PCR (Sanger, oxBS-seq, TAB-seq, NOME-seq)
- Bisulfite NGS library preparation (Whole Genome, RRBS)
- Pyrosequencing assays
- TA cloning

**EPiK Amplification Kit** has been specifically engineered for amplification of bisulfite-modified DNA and features a dedicated buffer system (EPiKORE™), designed to overcome the problems associated with bisulfite-modified, uracil-containing DNA templates such as template degradation and uracil stalling. EPiK Amplification Kit offers significant improvements in reliability, yield, sensitivity leading to far greater PCR success rates.

EPiK delivers truly unrivaled, market-leading performance, even with longer amplicons (1.5kb) (Fig. 1). Furthermore, EPiK offers significantly improved amplification success rates with low template concentrations (<0.5ng) of bisulfite-modified DNA (Fig. 2).

Powered by MyTaq™ HS and EPiKORE™ technology, the high speed and enhanced specificity of EPiK Amplification Kit makes it highly suited for high-throughput epigenetic assays and the very latest bisulfite sequencing applications.
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