

Thermo Scientific Advanced PCR Enzymes



Thermo Scientific Phusion High-Fidelity DNA Polymerase



Thermo Scientific Phusion Hot Start II High-Fidelity DNA Polymerase



Thermo Scientific Phire Hot Start II DNA Polymerase

PCR performance no other enzyme can match!

Thermo Scientific Phusion High-Fidelity DNA Polymerases

Since their introduction in 2003, Phusion® High-Fidelity DNA Polymerases have established a new standard for high-fidelity PCR. Phusion DNA Polymerases have proven first choice for several demanding PCR applications, including the creation of first functional synthetic genome^{1,2}.

In Phusion High-Fidelity DNA Polymerases, a DNA-binding domain is fused to a *Pyrococcus*-like proofreading polymerase. Due to this unique fusion technique, Phusion DNA Polymerases generate PCR products with accuracy and speed unattainable with a single enzyme, even on the most difficult templates. In addition, Phusion DNA Polymerases are tolerant of various PCR inhibitors. This allows robust amplification with minimal optimization. For hot start PCR, Phusion Hot Start II High-Fidelity DNA Polymerase is an ideal choice allowing extreme specificity and improved robustness.

The processivity* of Phusion DNA Polymerases is approximately 10-fold greater than that of *Pfu* DNA polymerase and twice that of *Taq* DNA polymerase. This extremely high processivity results in shorter extension times, more robust amplification and the ability to amplify long templates (up to 20 kb) in a fraction of the time. Phusion DNA Polymerases also produce higher yields with lower enzyme amounts than traditional proofreading polymerases.

Advantages

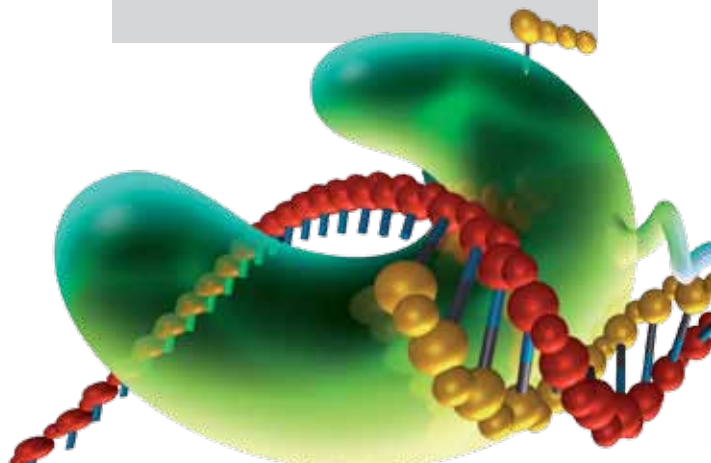
- Accuracy - The highest fidelity of any available thermostable polymerase
- Robustness - Fewer reaction failures and minimal optimization
- Speed - Increased processivity allows shorter reaction times (extension 15-30 s/kb)
- High Yields - Increased product yields with minimal enzyme amounts (0.5-1 U/50 µl reaction)
- Specificity - Unique hot start modification with zero-time reactivation reduces non-specific amplification and primer degradation

Applications

- High-fidelity PCR
- Fast PCR
- Hot Start PCR
- Long range PCR (up to 20 kb)
- High-throughput PCR

1. D.G. Gibson *et al.*, (2008) Complete chemical synthesis, assembly, and cloning of a *Mycoplasma genitalium* genome. *Science* 319, 1215-1220.
2. D.G. Gibson *et al.*, (2010) Creation of a bacterial cell controlled by a chemically synthesized genome. *Science* 329, 52-56.

* Processivity measures the number of nucleotides the enzyme can incorporate to a growing DNA strand at one binding event during the extension step.

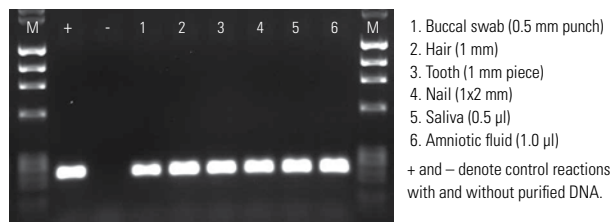


Extreme fidelity for demanding PCR

Phusion DNA Polymerases have extremely low error rates, thus setting a gold standard for high-fidelity PCR. The error rate, determined by a modified *lacI*-based method³, is approximately 50-fold lower than that of *Taq* DNA polymerase and 6-fold lower than that of *Pfu* DNA polymerase.

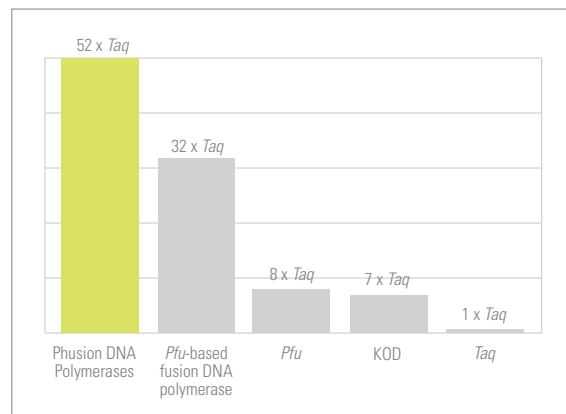
Minimize reaction failures

Phusion DNA Polymerases are exceptionally tolerant of inhibitors and other challenging reaction conditions. This provides reliability by minimizing reaction failures, and even enables PCR from unpurified sample materials. Phusion DNA Polymerases are the key components in Thermo Scientific Direct PCR Kits for human specimens and blood samples.

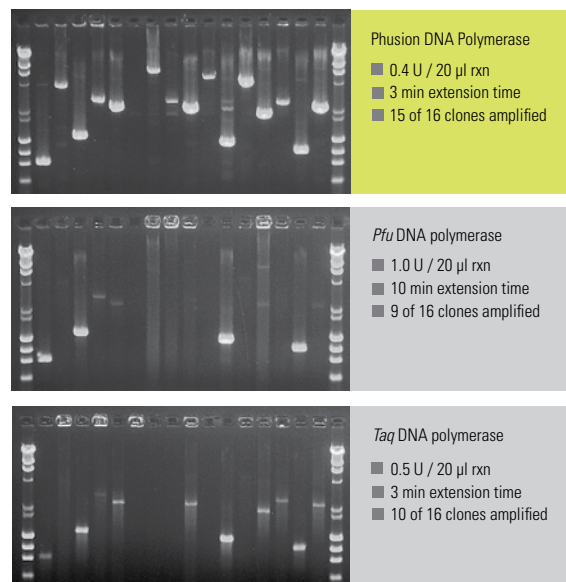


PCR from various human-derived samples without DNA purification

Different samples were placed directly into 20 μ l PCR reactions. A 237 bp DNA fragment was successfully amplified using the control primers included in the Phusion Human Specimen Direct PCR Kit. Reactions were run in Thermo Scientific UTW reaction vessels using Thermo Scientific Piko Thermal Cycler.



Relative fidelity values of different DNA polymerases.
Fidelity = 1 / error rate.



Phusion DNA Polymerase combines extreme fidelity with unparalleled speed and robustness

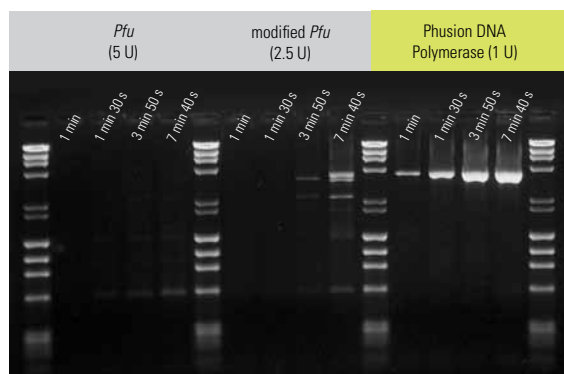
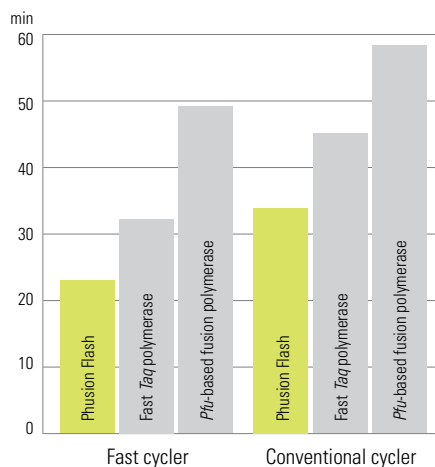
A random set of 16 clones from a *Thermus sp.* genomic library was amplified from bacterial colonies. The results highlight the robustness and speed of Phusion DNA Polymerase. It was able to amplify 94% of the amplicons with lower enzyme amounts still producing superior yields. The success rate of *Pfu* DNA polymerase was only 56% and that of *Taq* DNA polymerase 62% with significantly lower yields.

3. B.F. Frey & B. Suppmann (1995) Demonstration of the Expand PCR System's greater fidelity and higher yields with a *lacI*-based fidelity assay. *Biochemica* 2, 34-35.

Fast PCR with high fidelity

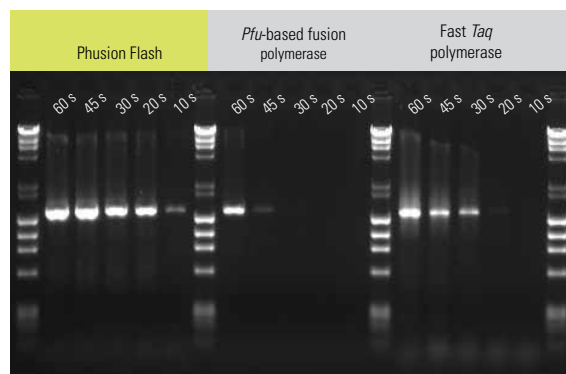
Phusion DNA Polymerases incorporate more nucleotides per binding event as compared to other polymerases. This high processivity allows extremely short extension times and consequently reduced protocol times. Shortest protocol times can be achieved with Thermo Scientific Phusion Flash High-Fidelity PCR Master Mix, a product developed especially for fast PCR. Due to the unique structure of the enzyme, Phusion DNA Polymerases are also highly efficient. When compared to conventional polymerases, significantly fewer units of the enzyme are required for any PCR reaction. Speed and efficiency result in high product yields in minimal time.

Shorter PCR run times with Phusion Flash



Less enzyme - superior yield

A 3.8 kb fragment from human beta globin gene was amplified with three different DNA polymerases. Phusion DNA Polymerase was able to amplify the 3.8 kb genomic fragment with a combined annealing and extension step of only 1 minute, thus being significantly faster than the two other polymerases tested. A single unit of Phusion DNA Polymerase produced higher yields than 2.5 or 5 units of the *Pfu* DNA polymerases.

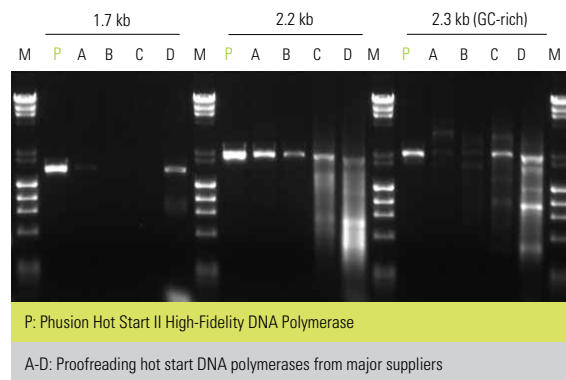


Extreme speed and high yields with Phusion Flash

A 1.5 kb human cathepsin K gene was amplified with three different polymerases using varying extension times (10-60 s) with Piko® Thermal Cycler. Only Phusion Flash was able to amplify the 1.5 kb gene with extremely short extension times of 10 and 20 s. It also produced superior yields of specific product compared to other enzymes tested.

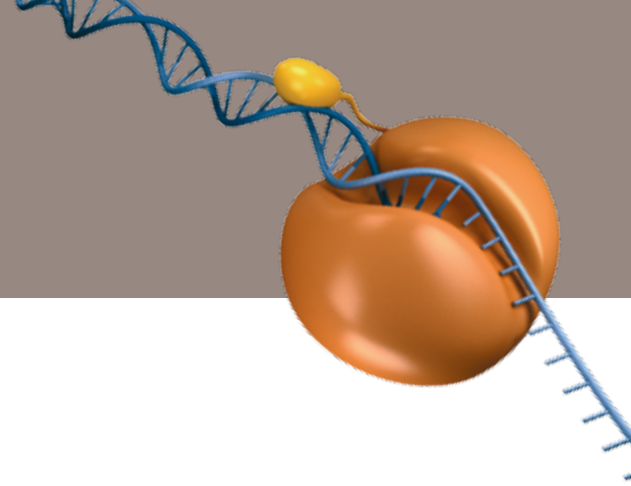
Extreme specificity with the hot start modification

The Affibody®-based inactivation method of the Thermo Scientific Phusion Hot Start II DNA Polymerase greatly increases the specificity of PCR amplification. Both the DNA polymerase and the proofreading activities of Phusion Hot Start II DNA Polymerase are inactivated at room temperature by a reversibly binding Affibody ligand. This prevents non-specific extension of the DNA template as well as degradation of the PCR primers during reaction setup. With Phusion Hot Start II DNA Polymerase, the reaction setup can be done at room temperature, enabling its use in high-throughput robotics. In addition to improved specificity, Phusion Hot Start II DNA Polymerase delivers improved robustness, allowing fewer reaction failures and minimizing the need for optimization.



Phusion Hot Start II DNA Polymerase provides extreme specificity and abundant yields. Five proofreading hot start DNA polymerases from major suppliers were used to amplify 1.7-2.3 kb fragments from human genomic DNA. Phusion Hot Start II DNA Polymerase provided high yields of specific products whereas all other enzymes delivered zero or low yields, with some also amplifying non-specific products.

Speed and specificity for routine applications



Thermo Scientific Phire Hot Start II DNA Polymerase

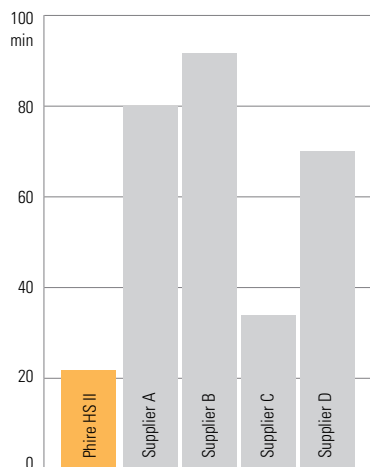
Phire® Hot Start II DNA Polymerase outperforms every *Taq*-based hot start polymerase on the market. This polymerase is significantly faster, extremely robust, and also capable of amplifying long DNA fragments with high yields. These features are achieved through advanced protein engineering of the polymerase. Phire Hot Start II DNA Polymerase incorporates a dsDNA-binding domain which allows short extension times (10-15 s/kb), improves yields, and increases fidelity 2-fold compared to *Taq* DNA polymerase. In addition, the unique hot start technology allows complete reactivation of the enzyme in “zero-time” at standard cycling temperatures. This combination of features makes Phire Hot Start II DNA Polymerase an ideal solution for routine and high throughput PCR applications. Phire delivers superior performance in conventional thermal cyclers as well as in fast instruments such as the Piko Thermal Cycler.

Advantages

- Quick hot start: No reactivation step
- Fast enzyme: Amplify 4 times faster than with hot start *Taq*
- Robust: Minimal reaction optimization due to high inhibitor tolerance
- High yields: Abundant products due to high efficiency
- Longer PCR products: Amplify significantly longer DNA fragments than with any hot start *Taq*

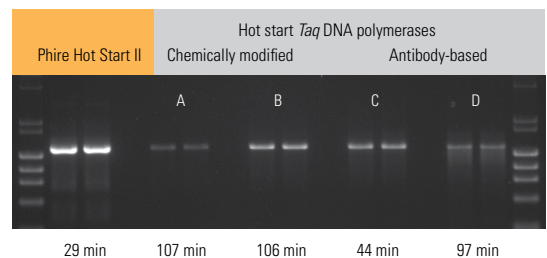
Applications

- Hot Start PCR
- Routine PCR
- Non-high fidelity PCR
- Fast PCR
- High-throughput PCR



Complete PCR protocols in less than half the time.

A 600 bp fragment from human genomic DNA was amplified with five different hot start DNA polymerases. With Phire Hot Start II DNA Polymerase, the PCR protocol was completed up to four times faster than with *Taq* DNA polymerases utilizing chemically modified or antibody-based hot start technologies (suppliers A-D).



Superior yields in significantly shorter time.

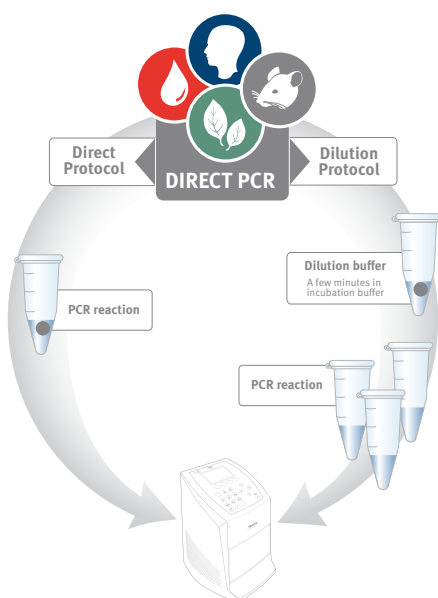
A 1.5 kb fragment from the human cathepsin K gene was amplified with five different hot start DNA polymerases. Phire Hot Start II DNA Polymerase amplified high amounts of specific PCR product in just 29 minutes. In contrast, the PCR protocols for hot start *Taq* DNA polymerases from four major suppliers (A-D) were substantially longer and resulted in lower product yields.

Related products

Thermo Scientific Direct PCR

Direct PCR kits deliver unprecedented convenience for DNA amplification. A tiny amount of sample is used directly in the PCR reaction with no prior purification steps. This allows significant savings in both time and cost. Direct PCR is based on Phusion and Phire DNA Polymerases, which are highly tolerant of PCR inhibitors present in unpurified sample materials. The following optimized kits are available:

- Phire Plant Direct PCR Kit
- Phire Animal Tissue Direct PCR Kit
- Phusion Human Specimen Direct PCR Kit
- Phusion Blood Direct PCR Kit



Thermo Scientific Phusion RT-PCR Kit

Phusion RT-PCR Kit is a complete two-step kit designed for high-fidelity RT-PCR. The kit contains the full set of reagents required for performing cDNA synthesis and PCR in two steps. The superior features of Phusion Hot Start II DNA Polymerase enable accurate cDNA amplification with high yields and short cycling times. Phusion RT-PCR Kit is an ideal choice when producing cDNA for cloning and gene expression studies.

Thermo Scientific Phusion Site-Directed Mutagenesis Kit

Phusion Site-Directed Mutagenesis Kit is a versatile and efficient tool for introducing point mutations, insertions or deletions in any type of plasmid DNA. The mutagenesis protocol comprises only three steps. Phosphorylated primers are designed to introduce the desired mutation(s) so that they anneal back to back to the plasmid. Phusion High-Fidelity DNA Polymerase amplifies the plasmid. The mutated plasmid is then circularized in a 5-minute ligation and transformed into bacteria.

HIGH PERFORMANCE PCR

The integrated solution for best results

High Performance PCR combines best-in-class DNA polymerases, PCR instruments and reaction vessels into a truly integrated solution for PCR. This powerful combination brings savings in time, space, and costs allowing DNA amplification with the highest accuracy and significantly shorter protocol times when compared to any other system.

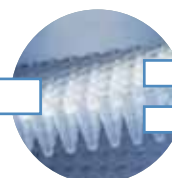


gDNA GUSB 0.7 kb A

Phusion High-Fidelity DNA Polymerases or Phire Hot Start II DNA Polymerase

Piko Thermal Cycler

UTW reaction vessels



Speed
Significantly faster than any other combination.

Fidelity
Phusion DNA Polymerases offer superior accuracy over *Taq* and *Pfu* based systems.

Yield
Higher amplification efficiency results in more product.

Specificity
Reduced levels of primer-dimers and false-primed products.

Enzyme characteristics and formats

		Phusion High-Fidelity DNA Polymerase	Phusion Hot Start II High-Fidelity DNA Polymerase	Phusion Flash High-Fidelity DNA Polymerase	Phire Hot Start II DNA Polymerase
CHARACTERISTICS	Blunt or 3'A end	Blunt	Blunt	Blunt	Blunt
	Target length	≤ 20 kb	≤ 20 kb	≤ 20 kb	≤ 7.5 kb
	Hot Start	No	Yes	Yes	Yes
	Recommended extension time	15-30 s/kb	15-30 s/kb	15 s/kb	10-15 s/kb
	Proofreading activity	Yes	Yes	Yes	Moderate
	Fidelity vs. <i>Taq</i>	52x	52x	25x	2x
AVAILABLE AS	Enzyme ¹	✓	✓	–	✓
	Master mix ²	✓	–	✓	–
	Complete kit ³	✓	–	–	–

1. Enzyme: DNA polymerase, buffer(s), DMSO and MgCl₂

2. Master mix: 2x master mix

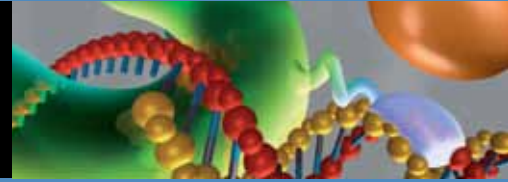
3. Complete kit: All the necessary PCR reaction components including control template and primers.

Ordering Information

Cat.No.	Product Description	Quantity
Phusion High-Fidelity DNA Polymerases, Master Mixes and Kits		
F-530S	F-530L Phusion High-Fidelity DNA Polymerase	100 U 500 U
F-531S	F-531L Phusion High-Fidelity PCR Master Mix with HF Buffer	100 x 50 µl rxns 500 x 50 µl rxns
F-532S	F-532L Phusion High-Fidelity PCR Master Mix with GC Buffer	100 x 50 µl rxns 500 x 50 µl rxns
F-548S	F-548L Phusion Flash High-Fidelity PCR Master Mix	100 x 20 µl rxns 500 x 20 µl rxns
F-553S	F-553L Phusion High-Fidelity PCR Kit	125 x 20 µl rxns 500 x 20 µl rxns
F-549S	F-549L Phusion Hot-Start II High-Fidelity DNA Polymerase	100 U 500 U
F-546S	F-546L Phusion RT-PCR Kit	20 rxns 100 rxns
F-541	- Phusion Site-Directed Mutagenesis Kit	20 rxns including 10 control rxns -
Phire Hot Start II DNA Polymerase		
F-122S	F-122L Phire Hot Start II DNA Polymerase	200 x 50 µl rxns (or 500 x 20 µl rxns) 1000 x 50 µl rxns (or 2500 x 20 µl rxns)
Direct PCR Kits		
F-130	- Phire Plant Direct PCR Kit	200 x 50 µl rxns -
F-140	- Phire Animal Tissue Direct PCR Kit	200 x 50 µl rxns -
F-150	- Phusion Human Specimen Direct PCR Kit	200 x 20 µl rxns -
F-547S	F-547L Phusion Blood Direct PCR Kit	100 x 20 µl rxns 500 x 20 µl rxns

In addition to Phusion and Phire PCR enzymes and kits, Thermo Scientific PCR portfolio includes other DNA polymerases for various purposes, PCR instruments and reaction vessels. For more information, please visit www.thermoscientific.com/pcrsolutions

Thermo Scientific Advanced PCR Enzymes



For further information visit:

www.thermoscientific.com/pcrsolutions

Technical Support:

US: techservice.genomics@thermofisher.com

Europe: techservice.emea.genomics@thermofisher.com

Ordering Information:

United States

Toll free: (800) 235-9880

Tel: +1 (303) 604-9499

Fax: +1 (303) 604-9680

Email: dharmacon.ordersassistance@thermofisher.com

Order online: www.thermoscientific.com/dharmacon

United Kingdom

Tel: +44 (0) 1372 723 456

Fax: +44 (0) 1372 741 414

Email: abgene.customerservices@thermofisher.com

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Germany

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US Literature # 00025265K01U
Euro Literature # PB_2010_94

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