

Amplification Products for PCR and RT-PCR

UptiTherm™ DNA Polymerase and kits

Selection guide

Polymerase	Hot start	Comment
UptiTherm™ DNA pol.	no	Most economic. Lower error rate than Taq polymerase Available in several formats, master mix including or not dNTP, Mg ²⁺ ..., in gel format (stable at room temperature)
UptiTherm™ EC DNA	no	for E.coli related gene amplifications
UptiTherm HotStart	yes	
taq Polymerase	no	For amplifications involving sequences homologous to E.coli, RADP
Red taq Polymerase	no	Visual control of activity
TEMPase polymerase	yes	Great for hot start

UptiTherm™ DNA Polymerase and kits

A superior polymerase from *Thermus thermophilus*

- ◆ Use for routine amplification of DNA
- ◆ Its error rate is 1.6-fold lower than that of *Taq* Polymerase
- ◆ Efficient for long range applications up to 5 Kbp

Applications :

UptiTherm™ DNA Polymerase is suitable for those applications requiring a highly thermostable enzyme capable of synthesising DNA at elevated temperatures, resolving the most complex secondary structures. The enzyme possesses a high processivity and a proven capacity to amplify DNA sequences weakly represented in complex mixtures (e.g. genomic DNA). Sequences can be detected in the reaction with initial quantities lower than 10 femtograms (0.01 picograms) of DNA.

Due to the high specificity of the enzyme, the recommended MgCl₂ concentration to use with UptiTherm™ DNA Polymerase is 2 mM. The standard MgCl₂ concentration is 2 mM. However, it is recommended to optimise this value in order to obtain the highest yield and specificity for each given experiment.

Radioactively labeled dNTP, as well as biotin, fluorescein, and digoxigenin-labeled dNTP (including dUTP) can be used as substrates.

The polymerase possesses terminal transferase activity, thus amplification products can be directly used for T/A cloning.

Polymerase activity remains unaltered after > 40 cycles of amplification.

Checked in a variety of applications, including plasmid amplification, genomic DNA amplification, bacterial colony screening, long amplifications from genomic or plasmid DNA (up to 5 Kbp-see Figure 1), multiplex amplifications, AFLP, etc.

Source and description :

Recombinant DNA polymerase from *Thermus thermophilus* HB27, cloned and purified from *E. coli* using non-chromatographic methods.

The purified enzyme possesses 5', 3' polymerase activity, as well as weak 5', 3' exonuclease activity. No 3', 5' exonuclease activity is detected.

Average error rate for UptiTherm™ DNA Polymerase is 1 error/10 Kbp (Taq polymerase error rate : 1/6Kbp).

The enzyme does not present significant reverse transcription activity.

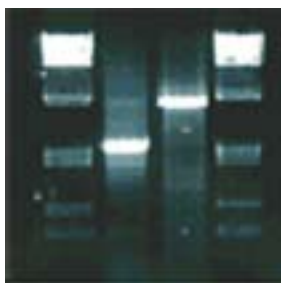
Half life at 94°C is 40 min.

For amplifications involving sequences homologous to *E. coli*

Quality control :

Each lot is carefully controlled to ensure the absence of non-specific endonucleases, as well as 3' 5' exonuclease and nicking activities. Lot to lot reproducibility is guaranteed. One unit of enzyme is defined as the amount necessary to incorporate 10 nmol of deoxynucleotide-triphosphates into acid-insoluble DNA within 30 min at 72°C.

Users may be required to obtain a license depending on the country and/or application.



Amplification performance for long range applications. Two DNA fragments of 3.5 and 5Kbp were amplified using UptiTherm™ DNA Polymerase for the amplification reaction, in a reaction volume of 50µl, including 10 pmoles of each primer in the supplied buffer, 200µM dNTP (Cat.No. UPS54211) and three (3) units of DNA Polymerase.

The amplification was performed after a previous phase of denaturation of 5 min at 94°C, 35 cycles of amplification (30 seconds at 94°C, 30 seconds at 55°C, 3 minutes at 72°C) and a final extension of 7 minutes at 72°C.

5µl of each amplification reaction were loaded in a 0.8% agarose MB gel (Cat.No. UPS54171).

See also Real-Time PCR kits page D31

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UptiTherm™ DNA Polymerase and kits

UptiTherm™ DNA Polymerase

A superior polymerase from *Thermus thermophilus*

UptiTherm™ DNA Polymerase is available in 2 concentrations :

- ◆ The concentration of 1U/μl has been designed for ease of use, and in order to avoid pipetting errors. Its use helps prevent inhibition/inespecificity of the reaction due to excess enzyme.
- ◆ The "traditional" concentration of 5 U/μl suits applications where reaction volumes are critical.

Description	Qty	1 U/μl	5 U/μl
UptiTherm™ DNA Polymerase			
Standard buffer (including MgCl ₂)	1000 U	UPS53663	UPS53881
Mg free buffer + MgCl ₂ , 50 mM	1000 U	UPS53703	UPS53921
Standard buffer (including MgCl ₂) + premixed dNTP (250 μl, 10 mM)	1000 U	UPS53733	UPS53763
Mg free buffer + MgCl ₂ , 50 mM + premixed dNTP (250 μl, 10 mM)	1000 U	UPS53793	UPS53823

Related products

Description	Cat.#
dNTP set (dATP, dGTP, dCTP, dTTP, 100 mM each)	UP968640
PCR Mix 3 (dATP, dGTP, dCTP, dTTP at 10 mM)	UP984440

See other "nucleotides" page D25

UptiTherm™ Master Mix in PCR tube

- ◆ "one tube – one reaction" format
- ◆ Less handling, less risk of contaminations: one vial with enzyme, MgCl₂, buffer and dNTPs
- ◆ Handling at room temperature
- ◆ Easy-going and fast : just add primers and DNA
- ◆ Hot Start : reagents do not interact until a temperature of 90°C is reached
Consumables saving

Description	Cat.#	Qty
UptiTherm™ Master Mix in PCR tube		
Standard Buffer with MgCl ₂ and dNTP (50 μl final reaction volume, including 200 μM dNTP, 1X buffer, 2 mM MgCl ₂ and 1U polymerase)	UPS54071	50 x 0.2 ml
Standard Buffer with MgCl ₂ and dNTP (50 μl final reaction volume, including 200 μM dNTP, 1X buffer and polymerase ; + 50 mM MgCl ₂ , vial)	UPS54081	50 x 0.2 ml

UptiTherm™ EC DNA-free Polymerase

- ◆ For amplifications involving sequences homologous to E.coli
- ◆ Recommended for RAPD
- ◆ Lot to lot reproducibility.

Recombinant DNA polymerase from *Thermus thermophilus* HB27, cloned and purified using non-chromatographic methods. The purified enzyme possesses 5' 3' polymerase activity, as well as weak 5' 3' exonuclease activity. No 3' 5' exonuclease activity is detected. Recommended for non-stringent applications (e.g. RAPD). UptiTherm™ EC is the enzyme of choice for applications involving bacterial DNA sequences homologous to those found in E. coli.

Average error rate for UptiTherm™ EC DNA-free Polymerase is 1 error/10Kbp. The enzyme does not present significant reverse transcription activity. Half-life at 94°C is 40 min.

UptiTherm™ EC DNA-free Polymerase is the UptiTherm™ enzyme with additional purification steps. It can be used the same applications as the standard UptiTherm™.

Description	Cat.#	Qty
UptiTherm™ EC DNA-free Polymerase		
1U/ml, Standard Buffer	UPS54141	500 U*
1U/ml, Mg free buffer + MgCl ₂	UPS54161	500 U*
Master Mix in PCR tube, standard buffer	UPS54051	50 x 0.2 ml vials
Master Mix in PCR tube, Mg free buffer (Polymerase + 50 mM MgCl ₂ in a separate vial)	UPS54061	50 x 0.2 ml vials



- 1 - Antibody coupled polymerase
- 2 - UptiTherm™ polymerase Master Mix in PCR tube
- 3 - Without hot-start
- 4 - Negative control

*One unit (1 U) of enzyme is defined as the amount necessary to incorporate 10 nmol of deoxynucleotide-triphosphates into acid-insoluble DNA within 30 min at 72°C.

Related products

Description	Cat.#
PCR DNA Marker (8 bands from 50 bp – 2 000 bp)	523920
PCR DNA Marker PLUS (19 bands from 11 bp – 2 000 bp)	N13990

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UptiTherm™ DNA Polymerase and kits

Taq DNA Polymerase

- ◆ Terminal transferase activity. Taq DNA polymerase has terminal transferase activity, which results in the addition of a single nucleotide (adenosine) at 3' end of the extension product.
- ◆ High-purity. No contamination activity has been detected in standard test reactions.

Taq DNA Polymerase is a thermostable DNA Polymerase isolated from a strain of *Thermus* sp. Taq DNA polymerase is the most common polymerase used for PCR reactions.

Taq Polymerase has been formulated using a proprietary technology, and the enzyme can be shipped at room temperature or stand at 37°C for 7 days without losing any activity.

Taq DNA polymerase can be used in most applications including the following :

- ◆ PCR*
- ◆ 3' A-tailing of blunt ends
- ◆ Primer extension
- ◆ DNA sequencing

* The PCR process is covered by US. Patent numbers 4683195 and 4683202 issued to Cetus and owned by Hoffman-La Roche Inc. We do not encourage or support the unauthorized use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license. Sale of this product is restricted in regions or countries where native Taq DNA polymerase patents have been invalidated. In any doubt, the UptiTherm is a Tth Polymerase that has never been affected by this patent, as the chemical properties, composition, production and purification methods used are totally different than those covered by the Roche patent.

Description	Cat.#	Qty
Taq DNA Polymerase (native), 5U/μl	GX5443	1000 U*

Supplied with 10 x reaction buffer containing 15 mM magnesium chloride. dNTP (10 mM) mixture need to be ordered separately.

Red Taq DNA Polymerase

- ◆ High performance thermostable DNA polymerase
- ◆ Red dye identifies tubes which contain enzyme and confirms complete mixing of reagents
- ◆ Ideal for TA Cloning

Red Taq DNA Polymerase is a thermostable recombinant DNA polymerase, which exhibits very high activity in primer extension and other molecular biology applications. Red Taq contains a red dye which provides easy and quick identification of reactions to which enzyme was added and allows confirmation of complete mixing. The inert dye has no effect on downstream processes. Red Taq is added directly to the reaction mix and is used in the same manner as standard Taq DNA Polymerase. Red Taq DNA Polymerase has both a 5' to 3' DNA polymerase and a double strand 5' to 3' exonuclease activity. The enzyme lacks a 3' to 5' exonuclease activity (no proofreading ability). Red Taq DNA Polymerase leaves an A overhang, which makes the enzyme ideal for TA cloning.

Description	Cat.#	Qty
Red Taq DNA Polymerase	UPAP1220	500 U*
5 U/μl, 10x Ammonium Buffer and MgCl ₂ (25 mM)	UPAP1221	1000 U*
	UPAP1222	2500 U*

* One unit (1 U) is defined as the amount that incorporates 10 nmoles of dNTPs into acid-precipitable form in 30 minutes at 72°C under standard assay conditions.

Amplification Products for PCR and RT-PCR

UptiTherm™ DNA Polymerase and kits

UptiTherm Hot Start PCR Master Mix

- ◆ Automated UptiTherm Hot Start enzyme for increased specificity and product yield
- ◆ Successful multiplex reactions saves time and reagents
- ◆ Designed to diminish the formation of non-specific product
- ◆ Detection of low target copy number

The Hot start PCR Kit is ideal for performing automated Hot Start PCR, a modification of the PCR process in which amplification reactions are initiated at an elevated temperature. During Hot Start, primers bind only to their specific target, and polymerase activity is directed exclusively to that target. As a result, only the region of interest is amplified, which increases sensitivity and yield while reducing non-specific background amplification. Hot start DNA Polymerase is essential for Hot Start PCR. A chemically modified form of the DNA Polymerase, facilitates the Hot Start process by means of its thermal activation property at a temperature well above optimal annealing. The enzyme remains inactive until the time, temperature, and pH are optimal. This improves specificity by prohibiting mis-priming and extension, thus eliminating waste and increasing yield. Because Hot start DNA Polymerase is a chemical Hot Start enzyme, there is no additional biological contamination. The Hot start DNA Polymerase also enables an unparalleled level of sensitivity. Sensitivity improves multiplex PCR, an applied PCR technique that amplifies several specific targets simultaneously. Applications that previously required two or more reactions can be performed in a single reaction tube. Hence, multiplexing represents a substantial savings of time and costly reagents.

Quality Control :

Endonuclease, exonuclease and priming activities are not detected after 3 hours incubation of 1 mg of pUC19 plasmid DNA and 0.5 µg EcoR I digested lambda phage DNA at 72°C in the presence of 40 units of Hot Start DNA Polymerase.

Description	10X Hot Start Buffer (MgCl ₂ 15 mM)	MgCl ₂ 50 mM	Size	Cat.#
UptiTherm Hot Start PCR Master Mix	1.5 ml	1.5 ml	250 U*	UPO6587A
	2 x 1.5 ml	2 x 1.5 ml	1000 U*	UPO6587B

Store at -20°C.

TEMPase™ Hot Start DNA Polymerases

Heat Activated

- ◆ Hot Start DNA Polymerase for increased specificity.
- ◆ Successful for multiplex reactions.
- ◆ Eliminates nonspecific products due to inactivity at room temperature
- ◆ Excellent specificity allows extension through DNA regions which contain repeats and secondary structures
- ◆ Leaves an "A" overhang

TEMPase utilizes a chemical modification to remain inactive at room temperature and works optimally in a specific buffer system.

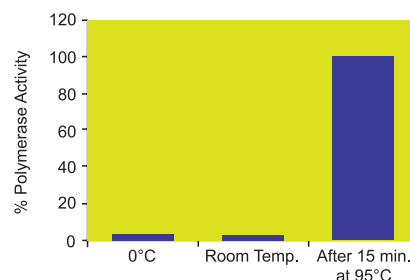
This enzyme is useful for performing automated hot start amplification due to the thermal activation at a temperature well above optimal annealing. The enzymes remain inactive until the time, temperature, and pH are optimal. During hot start, primers bind only to their specific target, and polymerase activity is directed exclusively to that target. As a result, only the region of interest is amplified, which increases sensitivity and yield while reducing non-specific background amplification.

Once the reaction reaches optimal annealing temperatures after a 15 minute activation step at 95°C, the enzyme stay activated in the reaction. Since the enzyme is chemically modified for hot start (no antibody), there is no material from animal sources, i.e., there is no possibility for biological contamination.

Description	Cat.#	Qty
TEMPase DNA Polymerase with reaction buffer II	UPBB3070	250 U*
	UPBB3071	500 U
	UPBB3072	1000 U
	UPBB3073	2500 U

* One unit (1 U) is defined as the amount that incorporates 10 nmoles of dNTPs into acid-precipitable form in 30 minutes at 72°C under standard assay conditions.

Heat Activation of TEMPase DNA Polymerase



TEMPase is inactive prior to heating at 95°C, thus eliminating the production of non-specific primer-template complexes.

- ◆ Storage Buffer : 20 mM Tris-HCl pH 8.5, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween[®] 20, 0.5% NP40, 50% glycerol.
- ◆ 10X Reaction Buffer II : Combination of (NH₄)₂SO₄, and KCl, 15 mM MgCl₂, 1% Tween 20.
- ◆ 25 mM MgCl₂ Solution
- ◆ Concentration : 5 units/µl.

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UptiTherm™ DNA Polymerase and kits

TEMPase™ Hot Start Master Mix

- ◆ Time saving
- ◆ Reduce contaminations
- ◆ Increase reproducibility

TEMPase Hot Start Master Mix is a ready-to-use 2 x master mix. Simply add primers, template and water to successfully carry out primer extensions and other molecular biology applications.

TEMPase Hot Start DNA Polymerase, the balanced K/NH₄⁺ buffer system, dNTPs and magnesium chloride are present in TEMPase Hot Start Master Mix with TEMPase Buffer II. Each reaction requires 25 µl of the 2 x reaction mix. Simply add primers, template and water to a total reaction volume of 50 µl.

TEMPase Hot Start Master Mix offers several advantages. Set up time is significantly reduced. The chance of contaminating component stocks is eliminated. Reduction of reagent handling steps leads to better reproducibility. Standard tests can be set up with the confidence that results will be consistent every time.

* One unit (1 U) is defined as the amount that incorporates 10 nmoles of dNTPs into acid-precipitable form in 30 minutes at 72°C under standard assay conditions.

Description	Cat.#	Qty
TEMPase DNA Polymerase Master Mix	UPBB2900	250 U*
	UPBB2901	500 U
	UPBB2902	1000 U
	UPBB2903	2500 U