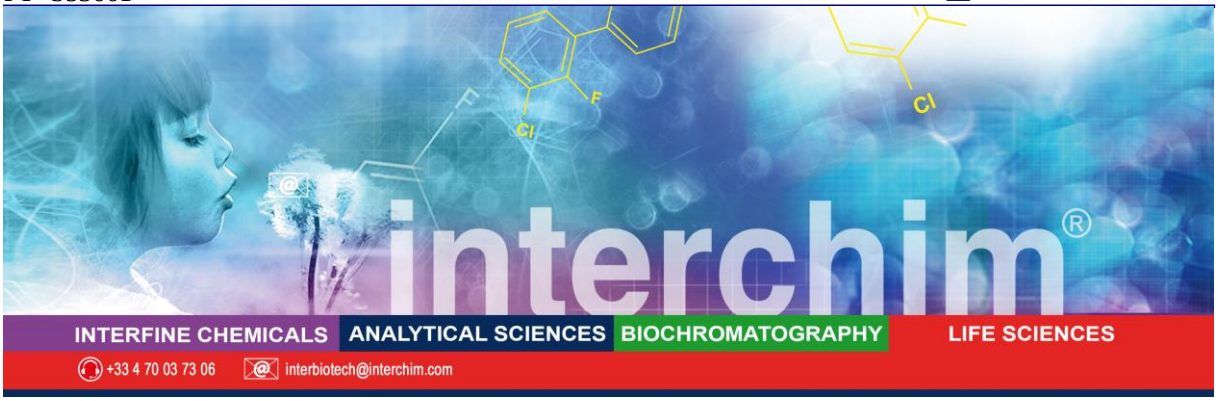
FT- S53661



UptiTherm DNA Polymerase

A superior polymerase with lower error rate (1/10 Kbp) to use for routine applications

Product Description

UptiTherm Product format (buffer)	Qty	Cat. No. 1 U/µl	Cat. No. 5 U/µl
Standard buffer (including MgCl2)	1000 U	UPS53663	UPS53881
Mg free buffer + $MgCl_2$ 50 mM	1000 U	UPS53703	UPS53921

Notes:
*1- UptiITherm DNA polymerase shows optimal specificity at **2 mM MgCl2** final concentration (rather than 1.5 mM) in reaction buffer.*
2- UptiTherm is also available as 500 U and 250 U package , and as a gel form (stable at room temperature!).

Store at - 20°C

Technical and Scientific Information

Highly thermostable DNA polymerase. It is a recombinant, modified form of the enzyme from the thermophilic bacterium Thermus thermophilus expressed in E. coli (see Note 1).

UptiTherm DNA polymerase is suitable for applications which require a highly thermostable and processive enzyme capable of synthesising DNA strands at elevated temperatures in amplification reactions or similar (e.g. primer extension), thus resolving the most complex secondary structures.

The enzyme is free of unspecific endo- or exonucleases activities, as well as nicking activities. It does not either exhibit significant reverse-transcriptase activity. Terminal transferase activity inherent to the enzyme renders A-tailed amplification products suitable to be further used in T/A cloning approaches.

The enzyme is supplied at a concentration of 5 U/µl and 1 U/µl in a storage buffer. 1 U/µl concentration allows accurate pipetting of small amounts of the DNA polymerase, so that it is not necessary to perform ulterior dilutions.

Unit Definition

One unit is defined as the amount of enzyme which incorporates 10 nanomoles of dNTPs into acid-insoluble DNA within 30 minutes at 72 ºC.

Storage buffer

10 mM Tris-HCl (pH 8.0), 50 mM KCl, 1 mM EDTA, 0.1% Triton X-100, 50% glycerol (v/v). Other storage buffers available (contact our Technical Dpt.).

Reaction buffer

Recommended reaction buffer is: 75 mM Tris HCl (pH 9.0), 2 mM MgCl2 (see Note 2), 50 mM KCl, 20 mM (NH4)2SO4. This reaction buffer (the so-called Standard Buffer, Ordering Information at the end) is supplied at 10X concentration together with the enzyme.

Reaction buffer can be supplied MgCl2 free (the so-called Free Buffer, see Ordering Information): Mg 2+ ion, being the enzyme cofactor, plays a key role on polymerase activity, this is why its concentration must be optimized in certain amplification-based experiments. In this case, the MgCl2 is supplied as a separate vial at 50 mM concentration. This solution must be completely thawed, vigorously vortexed and spun down in a bench-top centrifuge before use.

Storage Conditions

Store at -20ºC in a constant temperature freezer (i.e. do not use frost-free freezers). Under these conditions the activity of the enzyme remains unaltered over 18 months of storage. The glycerol in the storage buffer prevents freezing at -20ºC.

Reaction Conditions

After thawing the reaction buffer (and MgCl2 solution, in case the "free buffer" choice is adopted), shake all vials (buffer, enzyme, 50 mM MgCl2 solution) by gentle vortexing, later spin them down in a bench-top centrifuge, and eventually pipette desired volumes.

Keep all reagents on ice while they remain out of the -20ºC storage freezer, otherwise enzyme activity will decrease over the time. Wear disposable gloves and make use of sterile, DNase- and RNase-free pipette tips and tubes in order to avoid contaminations and false negative results.

Recommended enzyme volumes to be added to the reaction mix

Final reaction volumes	Recommended enzyme volumes	Volume using 1U/µl items (or a 1U/µl master mix)
100 µl	up to 2.5U	Up to 2.5 µl
50 µl	1-1.25U	1-1.25 µl
25 µl	0.5-0.75U	0.5-0.75 µl

These volumes may vary depending on the reaction conditions and on the particular nucleotide composition of the product to be amplified. Further information may be obtained from our Technical Department.

It is recommended to increase the enzyme units (up to three times) in order to perform certain applications such as PRINS (Primed In Situ Synthesis) or when working on long DNA fragment amplifications (longer than 2 Kb from genomic DNA).

The dNTP final concentration recommended is 200 µM (50 µM each), but this figure may be decreased (e.g. when unspecific amplimers occur), enlarged (e.g. long amplifications) or even unbalanced in favour of any dNTP in particular (e.g. in vitro mutagenesis experiments) depending on the intended approach. This enzyme also accepts modified dNTPs (e.g. radioactively or fluorescein labelled) as substrate.

List of UptiTherm products

Quantity	Standard Buffer 2 mM		Free $MgCl_2$ + 50 Mm $MgCl_2$	
	1 U/µl	5 U/µl	1 U/µl	5 U/µl
5000 units	S53665	S53885	S53705	S53925
1000 units	UPS53663	UPS53881	UPS53703	UPS53921
500 units	UPS53662	UPS53882	UPS53702	UPS53922

Notes

Note 1: this enzyme is not recommended for certain experiments dealing with amplification of sequences homologous to those found in E. coli or very low-annealing temperature amplification approaches (e.g. RAPDs, Random Amplified Polymorphic DNAs). This enzyme is not recommended for sequencing.

Note 2: at difference with the vast majority of the thermostable DNA polymerases existing in the market, UptiITherm DNA polymerase shows optimal specificity at 2 mM MgCl2 final concentration (rather than 1.5 mM) in reaction buffer.

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Related Products

- Mineral oil, #UPS5422A
- dNTP Mix (dATP, dGTP, dCTP, dTTP), #127823
- dNTP Set 1 (dATP, dGTP, dCTP, dTTP), #UP968640
- Mix Sequencing Term. (dNTP+ddNTP), #UP996780
- dNTP Set 2 (dATP, dGTP, dCTP, dUTP), #UP968660
- Agarose, regular uses, #31272L

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