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## UptiTherm EC DNA-free Polymerase

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### Product Description

Cat. No.	Product
UPS54140	100 units (1 U/ $\mu$ l) + Standard buffer (including 2 mM MgCl <sub>2</sub> )
UPS54141	250 units (1 U/ $\mu$ l) + Standard buffer (including 2 mM MgCl <sub>2</sub> )
UPS54160	100 units (1 U/ $\mu$ l) + Buffer MgCl <sub>2</sub> free + vial 50 mM MgCl <sub>2</sub>
UPS54161	250 units (1 U/ $\mu$ l) + Buffer MgCl <sub>2</sub> free + vial 50 mM MgCl <sub>2</sub>

Also available in gel form:

UPS54051	UptiTherm EC DNA Polymerase 50 x 0.2 ml vials, gel format, Standard buffer Including dNTPs
UPS54061	UptiTherm EC DNA Polymerase 50 x 0.2 ml vials, gel format, Mg free buffer Including dNTPs + vial MgCl <sub>2</sub> 50mM

#### Storage Conditions

Store at -20°C 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 -20°C. Gel form must be stored at 4°C.

## Scientific and Technical Information

#### Description

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

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

UptiTherm EC DNA-free Polymerase is also recommended for non-stringent applications (e.g. **RAPDs, Microarray**). It is the enzyme of choice for applications involving **bacterial DNA sequences homologous to those found in *E. coli***.

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 1 U/ $\mu$ l** in a *storage buffer*. This 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 °C.

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213 Avenue J.F. Kennedy - BP 1140  
03103 Montluçon Cedex - France  
Tél. 04 70 03 88 55 - Fax 04 70 03 82 60

[uptima@interchim.com](mailto:uptima@interchim.com), Hotline : 33(0)4 70 03 73 06

FT- UPS54140

## Reaction buffer

Recommended reaction buffer is: 75 mM Tris HCl (pH 9.0), **2 mM MgCl<sub>2</sub>** (see Note 1), 50 mM KCl, 20 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. This reaction buffer (the so-called Standard Buffer) is supplied at 10X concentration together with the enzyme (either accompanying it, or included in it, in the case of aliquoted vials).

Reaction buffer can be supplied MgCl<sub>2</sub> free (the so-called Free Buffer, see Ordering Information): Mg<sup>2+</sup> ion, being the enzyme cofactor, plays a key role on polymerase activity, this is why its concentration must be optimised in certain amplification-based experiments. In this case, the MgCl<sub>2</sub> 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.

## Reaction conditions

After thawing the reaction buffer (and MgCl<sub>2</sub> solution, in case the “free buffer” choice is adopted), shake all vials (buffer, enzyme, 50 mM MgCl<sub>2</sub> 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 -20°C 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 and units to be added to the reaction mix

Final reaction volumes	Recommended enzyme volumes
100 µl	Up to 2.5 µl
50 µl	1-1.25 µl
25 µl	0.5-0.75 µl

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, Cat. No UPS54211), 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.

## Notes

Note 1: at difference with the vast majority of the thermostable DNA polymerases existing in the market, UptiTherm EC DNA-free Polymerase shows optimal specificity at 2 mM MgCl<sub>2</sub> final concentration (rather than 1.5 mM) in reaction buffer.

*Notice to buyers/users :*

*Some of the applications which may be performed with this product are covered by applicable patents in certain countries.*

*The purchase of this product does not include or provide a license to perform patented applications.*

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

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213 Avenue J.F. Kennedy - BP 1140  
03103 Montluçon Cedex - France  
Tél. 04 70 03 88 55 - Fax 04 70 03 82 60

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[uptima@interchim.com](mailto:uptima@interchim.com), Hotline : 33(0)4 70 03 73 06