FT- UPAK510

UptiPfu DNA Polymerase

High fidelity amplification up to5 Kbp, involving a high number cycles (55-60). Ideal to increase the sensitivity of the reaction.

Product Description

Cat. No.	Product
UPAK5100	100 units (1 U/µl) + Standard buffer including $MgCl_2$
UPAK5101	250 units (1 U/µl) + Standard buffer including $MgCl_2$
UPAK5103	100 units (1 U/µl) + Mg free buffer + 50 mM $MgCl_2$ buffer
UPAK5104	250 units (1 U/µl) + Mg free buffer + 50 mM $MgCl_2$ buffer

Store at - 20°C (J)

Technical and Scientific Information

Highly thermostable DNA polymerase with proof-reading activity. It is a recombinant, modified form of the enzyme from the hiperthermophilic bacterium *Pyrococcus furiosus* expressed in *E. coli* (see Note 1).
UptiPfu DNA polymerase is suitable for applications which require a proof-reading, 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. *Pfu* DNA polymerase has an error rate of 1 x 10^{-6} (10-fold lower than non proof-reading DNA polymerases). After 1 hour of incubation at 94 ºC, the enzyme retains more than 90 % of its activity.
The enzyme is free of unspecific endonuclease activity, as well as nicking activities. It does not either exhibit nucleotidyl terminal transferase activity so its amplification products can be directly used for cloning in blunt-ended vectors.

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

20 mM Tris-HCl (pH 8.0), 50 mM KCl, 0,25% NP 40, 0,25% Tween 20, 40% glycerol (v/v).

Reaction buffer

Recommended reaction buffer is: 75 mM Tris HCl (pH 9.0), **2 mM $MgCl_2$** (see Note 2), 50 mM KCl, 20 mM $(NH_4)_2SO_4$. 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 $MgCl_2$ free: Mg^{2+} 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 $MgCl_2$ 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. **Nevertheless, should the enzyme become frozen, its activity is not altered.**

Reaction Conditions

After thawing the reaction buffer (and $MgCl_2$ solution, in case the "free buffer" choice is adopted), shake all vials (buffer, enzyme, 50 mM $MgCl_2$ 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.
Adding the Pfu DNA polymerase at the end is recommended to avoid oligonucleotides degradation because of its exonuclease activity (maximum if it is added in absence of dNTPs).
Recommended enzyme volumes to be added to the reaction mix
We recommend using 1-2 enzyme units (1-2 µl) for a final reaction volume of 50 µl. Change the amount of enzyme keeping this proportion if the final reaction volume is different. Each experiment may require the optimisation of the enzyme amount.
It is recommended to increase the enzyme units in order to amplify low DNA template concentrations or when working on long DNA fragment amplifications

Amplification program parameters

The amplification reaction parameters are similar to the ones for UptiTherm DNA polymerase (Cat. No UPS53921).
Nevertheless, and depending on the experiment, increasing the duration of the elongation step (72 ºC), up to twice maximum, may improve the efficiency of the reaction. Further information may be obtained from our Technical Department (interbiotech@interchim.com). Pfu has been tested in amplifications up to 5 Kbp (for amplifications of longer fragments, we recommend using a combination of Pfu and UptiTherm DNA Polymerase).

dNTPs.

The recommended final dNTP concentration is 200 µM (Cat. Nos UP926890, UP968640, UP984440), but this value may be decreased (e.g. when unspecific amplimers occur), increased (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. In case dNTP concentration is modified, it may be necessary to optimise $MgCl_2$ accordingly.

Notes

Note 1: this enzyme is **not** recommended for a number of 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).
Note 2: at difference with the vast majority of the thermostable DNA polymerases existing in the market, UptiPfu DNA polymerase from UptiTherm shows optimal specificity at 2 mM $MgCl_2$ final concentration (rather than 1.5 mM) in reaction buffer.