KlearTaq HiFi DNA Polymerase

(For research use only. Not for use in diagnostic procedures.)

****Please ensure that the kit is stored at -20°C****

Introduction

KlearTaq™ HiFi DNA polymerase is unique blend of KlearTaq DNA polymerase and a proof-reading polymerase with 3'-5' nuclease activity. The addition of a proof-reading polymerase to KlearTaq results in a blend that has the high fidelity associated with proof-reading enzymes and the speed of KlearTaq polymerase.

KlearTaq is a highly specific, robust and efficient enzyme that is suitable for the majority of PCR applications and is produced by over-expression of the Taq DNA polymerase gene cloned into an *E. coli* vector.

KlearTaq HiFi enzymes are highly purified by a combination of differential thermal denaturation, size exclusion and ion exchange chromatography. Post purification, the enzyme is inactivated by a novel method (patent in preparation), resulting in an enzyme that gives highly specific and robust performance in PCR.

KlearTaq HiFi is designed for use in applications requiring very low error rates such as DNA sequencing, cloning experiments, and amplification of large amplicons. It can be used with templates such as BACs, cosmids, λ clones, and high molecular weight DNA.

KlearTaq HiFi is suited to the following applications:

- Generation of amplicons over 10 kb on genomic templates
- Generation of amplicons over 40 kb on less complex DNA
- Sequencing
- Cloning
- Standard PCR of genomic, viral and plasmid templates.

Kit contents

Each KlearTaq HiFi DNA polymerase kit is supplied with an optimised reaction buffer (10X). This buffer contains magnesium (1.8 mM final concentration). A separate tube of 50 mM $MgCl_2$ is included for further optimisation.

Customer requirements

1. dNTP mix
2. Nuclease-free water
3. Forward (upstream) primer
4. Reverse (downstream) primer
5. Template DNA.

General guidelines

- KlearTaq HiFi polymerase requires a 15 minute initial activation stage (94°C) prior to PCR.
- The annealing step can be optimised, taking the calculated melting temperature of the primers into consideration.
- For the first 20 cycles, allow a 1 minute extension (68°C) for every 1 kb of DNA to be amplified. For the remaining 15 cycles, allow a 2 minute extension (68°C) for every 1 kb of DNA to be amplified.
- A final extension step of 20 minutes at 72°C is recommended.

Reaction set-up

The PCR setup detailed in Table 1 is intended for guidance only. Conditions will vary for different PCR reactions and may require optimisation.

Table 1: Example PCR setup using KlearTaq HiFi enzyme

Component	Final concentration	20 µL reaction	50 µL reaction
10x buffer	1x	2 µL	5 µL
dNTPs (2.5 mM each)	500 µM	4 µL	10 µL
Forward primer (100 µM)	0.8 µM	0.16 µL	0.4 µL
Reverse primer (100 µM)	0.8 µM	0.16 µL	0.4 µL
KlearTaq HiFi (5 units / µL)	2.5 units per 50 µL	0.2 µL	0.5 µL
$MgCl_2$ (50 mM)	2.5 mM	0.28 µL	0.7 µL
Template DNA	-	As required	As required
Water	-	to 20 µL	to 50 µL
Total (µL)	-	20	50

Protocol

1. Completely thaw all of the reaction components and briefly vortex before use. Briefly spin the tubes in a microcentrifuge to ensure that the material is collected at the bottom of the tube. Ensure that the KlearTaq enzyme is stored on ice throughout reaction setup. **Please note:** LGC recommend that a mastermix is prepared rather than attempting to pipette small volumes of each of the reaction components for each PCR reaction.
2. In a sterile, nuclease-free microcentrifuge tube combine all components of the PCR reaction. Work on ice.
3. Briefly spin the reaction tubes in a microcentrifuge to ensure that the material is collected at the bottom of the tube.
4. Place the reactions in a thermal cycle and perform the PCR reaction according to parameters in Table 2.

Table 2: Thermal cycling conditions for PCR using KlearTaq HiFi

Step	Temperature	Time	Number of cycles
1	94	15 min	1 cycle
2	94	30 sec	20 cycles
	57	30 sec	
	68	1 min / kb	
3	94	30 sec	15 cycles
	57	30 sec	
	68	2 min / kb	
4	72	20 min	1 cycle

Further information about KlearTaq HiFi

KlearTaq is a 94 kDa, recombinant thermostable DNA polymerase from the thermophilic bacterium Thermus aquaticus, obtained by high-level expression of the Taq DNA polymerase gene in *E. coli*.

- KlearTaq HiFi polymerase exhibits optimal activity at 75°C and has a half-life of approximately 45 min at 94°C.
- KlearTaq is inactivated using LGC's proprietary method. The activation completely prevents non-specific primer annealing and the formation of primer dimers during setup.
- The Klear Taq HiFi blend has an error rate of approximately 4.0 x 10^{-6} errors per nucleotide incorporation event.

Ordering information

Product code	Product name	Description
KBS-1000-101	KlearTaq HiFi 500	100 µL, supplied at 5 units / µL
KBS-1000-102	KlearTaq HiFi 1000	200 µL, supplied at 5 units / µL
KBS-1000-103	KlearTaq HiFi 5000	1 mL, supplied at 5 units / µL
KBS-1000-104	KlearTaq HiFi 50000	10 mL, supplied at 5 units / µL

Unit definition: One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid-insoluble form in 30 minutes at 72°C.

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