KlearTaq DNA Polymerase

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

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

Introduction

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

The enzyme is 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 is suited to the following applications:

- Standard PCR of genomic, viral, and plasmid templates
- DNA labelling
- TA cloning.

Kit contents

Each KlearTaq DNA polymerase kit is supplied with an optimised reaction buffer (10X), 50 mM $MgCl_2$ 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

1. KlearTaq requires a 15 minute initial denaturation stage (95ºC).
2. The annealing step can be optimised, taking the calculated melting temperature of the primers into consideration.
3. Allow a 1 minute extension (72ºC) for every 1 kb of DNA to be amplified.
4. A final extension step of 5 minutes at 72ºC is recommended.

Reaction set-up

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

Table 1: Example PCR set-up using KlearTaq enzyme

Component	Final concentration	20 µL reaction	50 µL reaction
10x buffer	1x	2 µL	5 µL
dNTPs (2.5 mM each)	250 µM	2 µL	5 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 (5 units / µL)	1 unit per 50 µL	0.08 µL	0.2 µL
Template DNA	-	as required	as required
Nuclease-free water	-	to 20 µL	to 50 µL
Total (µL)	-	20 µL	50 µL

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

Step	Temperature	Time	Number of cycles
1	95	15 min	1 cycle
2	95	30 sec	34 cycles
	61	30 sec	
	72	1 min / kb	
3	72	5 min	1 cycle

Further information about KlearTaq

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 polymerase exhibits optimal activity at 75ºC and has a half-life of approximately 45 min at 94ºC.
- KlearTaq also demonstrates 3’ adenylation activity; hence the resulting PCR product is suitable for effective integration into TA cloning vectors.
- 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 fidelity of KlearTaq is approximately 2.2 x 10^{-5} errors per nucleotide incorporation event, or 4.5 x 10^{4} nucleotides incorporated before an error occurs.
- The enzyme has a 5’ - 3’ polymerisation-dependent exonuclease replacement activity but lacks a 3’ - 5’ nuclease activity and therefore does not have a proof-reading function.

Ordering information

Product code	Product name	Description
KBS-1000-001	KlearTaq 500	100 µL, supplied at 5 units / µL
KBS-1000-002	KlearTaq 1000	200 µL, supplied at 5 units / µL
KBS-1000-003	KlearTaq 5000	1 mL, supplied at 5 units / µL
KBS-1000-004	KlearTaq 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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For any queries about this guide please contact:
All locations except USA: email tech.support@lgcgenomics.com or call +44 (0)1992 476 486
USA only: email us-support@lgcgenomics.com or call +1 978 338 5317