

Products	Cat #	Pack Size
KlenTherm™ DNA Polymerase	GC-001-0250	250 u
KlenTherm™ DNA Polymerase	GC-001-0500	500 u
KlenTherm™ DNA Polymerase	GC-001-1000	1000 u
KlenTherm™ DNA Polymerase	GC-021-5000	5000 u

DESCRIPTION

KlenTherm™ DNA Polymerase is thermostable polymerase corresponding to the KlenTaq Polymerase described by W. M. Barnes. It is a N-terminally truncated Taq DNA polymerase. As expressed from a gene construct in E.coli, translation initiates at Met236, bypassing the 5'-3' exonuclease domain of the DNA polymerase encoding gene. This deletion leaves a **highly active** and even **more heat-stable** DNA polymerase activity. **Repeated exposure to 98°C does not seem to diminish the enzyme activity.** Significant activity remains even after exposure **to 99°C**. The full length enzyme does not tolerate these treatments. Therefore KlenTherm™ DNA polymerase is an **excellent alternative to modified T7 RNA polymerase** in thermal sequencing methods. Even problematic DNA templates with **secondary structures** and **GC-rich regions** can be **sequenced at 70°C**.

You can use KlenTherm™ DNA polymerase also for **Long-PCR up to 35 kb** in combination with thermostable „proof-reading“ polymerases (e.g. AccuTherm™). GeneCraft offers several mixtures of KlenTherm™ DNA polymerase called **Synergy™**.

In special applications KlenTherm™ DNA polymerase has proven better specificity than regular Taq polymerase. This results in minimising of unspecific DNA amplification products.

KlenTherm™ DNA polymerase is similar to USB Taq and **Cetus Stoffel fragment**. You will need more KlenTherm than Taq protein if the nucleic acid incorporation is more than 500 bp. KlenTherm™ DNA polymerase is shipped at higher (10 u/μl) concentration, so that it can easily incorporate 2 kb, if the same quantity is used as for full-length Taq. The use of KlenTherm™ is especially recommended for **amplifications of small fragments from genomic DNA**.

KlenTherm has a very low 3'-A-Overhang-adding activity.

APPLICATION

- **Fidelity** The relative mutation rate during polymerization is twofold lower for KlenTherm as compared to the full-length Taq DNA polymerase.
- **Cycle sequencing** The absence of the 5'-3' exonuclease activity makes KlenTherm™ especially suitable for cycle sequencing. It gives higher sequence intensity and very low backgrounds.
- **Long PCR** KlenTherm™ in combination with a Pfu DNA polymerase (AccuTherm) exhibiting a proof-reading activity can amplify up to 35 kb DNA fragments (pozri typ **Synergy™**).
- **Mutation analysis** KlenTherm™ has a reduced tendency to extend a mismatched 3'-oligonucleotide end making it suitable for mutation analysis with mutationspecific oligos (ARMS analysis).

CONCENTRATION

10 units/ μ l

UNIT DEFINITION

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid insoluble form in 30 min at 72°C under the assay conditions 25 mM TAPS (tris-(hydroxy-methyl)-methyl-amino-propanesul-fonic acid, sodium salt) pH 9.3 (at 25.C), 50 mM KCl, 2 mM MgCl₂, 1 mM .-mercaptoethanol) and activated calf thymus DNA as substrate.

STORAGE BUFFER

10 mM K-phosphate buffer pH 7,0; 100 mM NaCl; 0,5 mM EDTA; 1 mM DTT; 0,01% Tween 20; 50% glycerol (v/v)

STORAGE TEMPERATURE

Store KlenTherm™ DNA polymerase below 0°C, preferably at -20°C, in a constant temperature freezer.

10 x REACTION BUFER

500 mM KCl, 100 mM Tris-HCl (pH 9 at 25°C), 1% Triton X100

Extra solution: 50 mM MgCl₂, add MgCl₂ to a final concentration of 3.5 mM.

Please note the difference between KlenTherm™ and BioTherm™ reaction buffers!

Cat. No **GC-001-006** 1.5 ml 10x reaction buffer

AMPLIFICATION CONDITIONS (for fragment cca 1,5 kb)

- | | |
|--|-------------------------------|
| • 10x reaction buffer | 3 μl |
| • 50 mM MgCl ₂ | 2.1 μl |
| • dNTP Mix10 (end concentration 200 μ M) | 0.6 μl |
| • human genomic DNA (300-600 ng) | 1 μl |
| • forward primer (25 pM) | 2 μl |
| • reverse primer (25 pM) | 2 μl |
| • KlenTherm™ (10 units/ μ l) | 0.5 μl |
| • H ₂ O | 18.8 μl |

total 30 μ l

- | | |
|---------------|---------------|
| • 58°C | 30 sec |
| • 72°C | 2 min |
| • 93°C | 20 sec |

30 cycles