

## DATA QUALITY SHEET



### **KlenTherm™ DNA Polymerase**

FOR RESEARCH USE ONLY

*Cat. GC-001-0100, GC-001-0250, GC-001-0500, GC-001-1000, GC-001-5000*

#### **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, in the recommended reaction buffer, 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. KlenTherm™ DNA polymerase is similar to, yet distinct from, 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.

The 10x reaction buffer (on request with or without MgCl<sub>2</sub>) is delivered free of charge. KlenTherm has a very low 3'-A-Overhang-adding activity.

#### **CONCENTRATION**

10 units/μl.

#### **UNIT DEFINITION**

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acidinsoluble 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 MgCl<sub>2</sub>, 1 mM β-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)

#### **REACTION BUFFER**

500 mM KCl, 100 mM Tris-HCl (pH 9 at 25°C), 1% Triton X100  
Extra solution: 50 mM MgCl<sub>2</sub> , add MgCl<sub>2</sub> to a final concentration of 3.5 mM.  
Please note the difference between KlenTherm™ and BioTherm™ reaction buffers!  
1.5 ml 10x reaction buffer Cat. No **GC-001-006**

#### **STORAGE TEMPERATURE**

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

#### **AMPLIFICATION CONDITIONS**

- 10x reaction buffer 3  $\mu$ l
- 50 mM MgCl<sub>2</sub> 2.1  $\mu$ l
- dNTP Mix10 (end concentration 200  $\mu$ M) 0.6  $\mu$ l
- human genomic DNA (300-600 ng) 1  $\mu$ l
- forward primer (25 pM) 2  $\mu$ l
- reverse primer (25 pM) 2  $\mu$ l
- KlenTherm<sup>TM</sup> (10 units/ $\mu$ l) 0.5  $\mu$ l
- H<sub>2</sub>O 18.8  $\mu$ l

**total 30  $\mu$ l**

- 58°C 30 sec
- 72°C 2 min
- 93°C 20 sec
- 30cycles
- Fragment is about 1.5 kb

#### **SHELF LIFE**

2 years from date of receipt under proper storage conditions.

#### **FUNCTIONAL ANALYSIS**

Tested functionally in a unit activity test