KlenThermase™ DNA Polymerase

Cat. GC-018-1000

DESCRIPTION

KlenThermase™ DNA polymerase is an optimised version of Taq DNA polymerase designed for cycle sequencing with dideoxynucleotides. This enzyme is recommended both for manual DNA sequencing with ^{35}S label and for automated fluorescent DNA sequencing. Mutations have been introduced into the active site of KlenThermase™ DNA polymerase that improve its ability to confer the extremely high enzymatic specificity and high fidelity incorporation of deoxy- and dideoxynucleotides. KlenThermase™ is recommended for SNP genotyping by allele-specific PCR (AS-PCR), allele-specific primer extension (AS-PEX) and mini-sequencing procedures.

CONCENTRATION

25 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 under the assay conditions (25 mM TAPS (tris-(hydroxymethyl)-methyl-aminopropane-sulfonic acid, sodium salt) pH 9.3 (at 25°C), 50 mM KCl, 2 mM $MgCl_2$, 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).

REACTION BUFFER

500 mM KCl, 100 mM Tris-HCl (pH 9 at 25°C), 1% Triton X100.
Extra solution: 50 mM $MgCl_2$, add $MgCl_2$ to a final concentration of 3.5 mM.
Not provided.

STORAGE TEMPERATURE

Store KlenThermase™ DNA polymerase below 0°C, preferably at -20°C, in a constant temperature freezer. Avoid repeated freeze-thaw.

SHELF LIFE

18 months from date of receipt under proper storage conditions (-20 ^{o}C)

FEATURES

Fidelity: The relative mutation rate during polymerisation is twofold lower for KlenThermase™ as compared to the full-length Taq DNA polymerase.

Cycle sequencing: The absence of the 5'-3' exonuclease activity makes KlenThermase™ especially suitable for cycle sequencing. It gives higher sequence intensity and very low backgrounds. The mutational optimization improves the uniformity of band intensities. Combination of KlenThermase™ with Tth inorganic pyrophospatase generates uniform bands that improve sequencing accuracy and give long read lengths.

CONTENTS

KlenThermase™ DNA polymerase (25 U/µl),	1000 Units

REFERENCE:

1. Minisequencing protocol: Lovmar L., etc, Quantitative evaluation by minisequencing and microarrays reveals accurate multiplexed SNP genotyping of whole genome amplified DNA. Nucleic Acids Res. 31: e129, 2003.

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2. Schnorrer, F., Ahlford, A., Chen, D., Milani, L. and Syvänen, A.-C. Positional cloning by fast-track SNP-mapping in Drosophila melanogaster. Nature Protocols 3, 1751 – 1765, 2008

3. Ahlford, A., Kjeldsen, B., Reimers, J., Lundmark, A., Romani, M., Wolff, A., Syvänen, A.-C. and Brivio, M. Dried reagents for multiplex genotyping by tag-array minisequencing to be used in microfluidic devices. Analyst,135, 2377-2385, 2010

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