



KAPATaq DNA Polymerase

Technical Data Sheet

Product Code		Kit Size
Without Dye	With Dye	
KK1014	KK1020	250 units
KK1015	KK1022	500 units
BK1000	BK1004	2500 units
BK1002	BK1006	5000 units

Product Description

KapaTaq is the single-subunit *Taq* DNA polymerase enzyme from the thermophilic bacterium *Thermus aquaticus*, purified from recombinant *Escherichia coli*. *Taq* polymerizes DNA from a primer annealed to a DNA template in the presence of deoxyribonucleoside triphosphates. *Taq* possesses 5'→3' polymerase activity, as well as double-strand dependent 5'→3' exonuclease activity. The enzyme lacks 3'→5' exonuclease activity and therefore does not possess a proofreading function.

The fidelity of *Taq* is in the range of 2.5×10^{-5} errors per nucleotide incorporation event, or an average of 4.0×10^4 nucleotides incorporated before an error occurs.

KapaTaq is available with loading dye reaction buffer. After cycling you can load your PCR product directly onto the agarose gel.

Applications

KapaTaq is suited to the following applications:

- Standard PCR
- DNA labeling
- DNA sequencing
- Numerous applications for which a high-quality thermostable DNA polymerase is required

Specifications

Concentration: 5 U/μl

Unit definition: One unit is defined as the amount of activity that will incorporate 10 nmol of nucleotides into acid-insoluble material in 30 minutes at 72°C¹.

Quality Control

KapaTaq is extensively purified, and is free of contaminating exonuclease and endonuclease activities. Each batch of KapaTaq is confirmed > 98 % pure by electrophoresis on an Agilent Bioanalyzer 2100 Protein230 assay. KapaTaq meets strict requirements with regard to DNA contamination.

Kit Components

- KapaTaq DNA Polymerase (5 U/μl in storage buffer)
- 10x High Yield Reaction Buffer with Mg²⁺ (Buffer A) with or without dye. KapaTaq w/loading dye includes only Buffer A.
- 10x Standard Reaction Buffer with Mg²⁺ (Buffer B)
- 25 mM MgCl₂

Storage

Store all components at -20°C

Standard PCR Protocol

Notes:

- The KapaTaq reaction buffers already contain Mg^{2+} at 1.5 mM final concentration. Supplement with additional $MgCl_2$ only if required.
- KapaTaq polymerase can be used in place of any other commercial Taq polymerase in an existing protocol. Take care to match reaction buffer and Mg^{2+} concentration as closely as possible.

Standard PCR reactions might consist of:

	Final conc.	20 μ l rxn	50 μ l rxn
PCR grade water		up to 20 μ l	up to 50 μ l
10x buffer with Mg^{2+}	1 x	2 μ l	5 μ l
dNTPs (10 mM)	200 μ M	0.4 μ l	1 μ l
Fwd Primer (10 μ M)	0.4 μ M	0.8 μ l	2 μ l
Rev Primer (10 μ M)	0.4 μ M	0.8 μ l	2 μ l
Template		as required	as required
KapaTaq (5 U/ μ l)	1 U per 50 μ l	0.08 μ l	0.2 μ l

Cycling instructions

Initial Denaturation:	95 °C	2 min
Denaturation:	95 °C	30 s
Annealing:	$T_m - 5$ °C	30 s
Extension:	72 °C	1 min/kb
Final extension:	72 °C	2 min

25 – 35 cycles

Notes on Cycling Conditions

- Initial denaturation can be performed over an interval of 1-5 min at 95°C depending on the GC content of template.
- Denaturation for 30 secs to 2 mins at 94-95°C is sufficient. If the amplified DNA has a very high GC content, denaturation time may be increased up to 3-4 min.
- Optimal annealing temperature is 5°C lower than the melting temperature of primer-template DNA duplex. If nonspecific PCR products are obtained optimization of annealing temperature can be performed by increasing temperature stepwise by 1-2°C.
- The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR product. 25-35 cycles are usually sufficient for the majority PCR reactions. Low amounts of starting template may require 40 cycles.
- The time of the final extension step can be extended for amplicons that will be cloned into T/A vectors.

References

1. Schleper, C. et al., Characterization of a DNA polymerase from uncultivated psychrophilic archaeon *Cenarchaeum symbiosum* (1997) J. Bacteriol., 179, 24, 7803-7811

Product warranty and licensing information can be found at: www.kapabiosystems.com

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