KAPABIOSYSTEMS

Technical Data Sheet

KAPA Taq PCR Kit with Mg-free Buffers

KR0353 – v6.16

Product Description

KAPA Taq DNA Polymerase is the single-subunit *Taq* DNA polymerase of the thermophilic bacterium *Thermus aquaticus*, purified from recombinant *Escherichia coli*. KAPA Taq DNA Polymerase has 5'→3' polymerase and 5'→3' exonuclease activity, but no 3'→5' exonuclease (proofreading) activity. The enzyme system has an error rate of approximately 1 error per 2.2 x 10^5 nucleotides incorporated. PCR products generated with KAPA Taq are A-tailed and are suitable for cloning into TA cloning vectors.

KAPA Taq Buffer C is a uniquely-formulated *Taq* buffer that facilitates specific primer annealing. This translates to higher yields of specific product when compared with traditional *Taq* buffers, and improved amplification of GC- and AT-rich templates. KAPA Taq Buffer D is a standard Tris-ammonium sulphate-based buffer, which is recommended for amplification of fragments >1 kb in size. Both are 5X buffers, supplied without $MgCl_2$ for optimal flexibility. KAPA Taq DNA Polymerase may, however, be used in combination with any standard *Taq* buffer with a pH of 8.3 or higher.

Product Applications

KAPA Taq DNA Polymerase is ideally suited for:

- Routine PCR
- DNA labelling
- Amplification of DNA for Sanger sequencing
- Any standard PCR application for which a high-quality thermostable DNA polymerase is required.

Product Specifications

Shipping and Storage

KAPA Taq PCR kits are shipped on dry ice or ice packs, depending on the country of destination. Upon arrival, store kit components at -20°C in a constant-temperature freezer. When stored under these conditions and handled correctly, full activity of the kit is retained until the expiry date indicated on the kit label. KAPA Taq Mg-free Buffers contain isostabilizers and may not freeze solidly, even when stored at -20°C. This will not affect the shelf-life of the product.

Kapa/*Roche* Kit Codes and Components	
KK1043 *07958579001* (250 U) **KK1040** *07958587001* (500 U)	KAPA Taq DNA Polymerase (5 U/µL) KAPA Taq Buffer C (10X) KAPA Taq Buffer D (10X) $MgCl_2$ (25 mM)

Quick Notes

- KAPA Taq DNA Polymerase can replace any commercial *Taq* DNA polymerase in an existing protocol. The final $MgCl_2$ concentration may need to be optimized to account for differences in buffer formulation.
- KAPA Taq Buffer C is a uniquely formulated buffer offering improved specificity and sensitivity, and is recommended for routine amplification of amplicons <1 kb.
- KAPA Taq Buffer D is recommended for amplicons >1 kb.
- Both buffers may be evaluated to determine the buffer most suitable for a specific application.
- The KAPA Taq PCR system is suitable for the amplification of fragments up to 3.5 kb from genomic DNA or 5 kb from less complex targets.

Handling

Always ensure that the product has been fully thawed and mixed before use. Reagents may be stored at 4°C for short-term use (up to 1 month). Return to -20°C for long-term storage.

Quality Control

Each batch of KAPA Taq DNA Polymerase is confirmed to contain <2% contaminating protein (Agilent Protein 230 Assay). KAPA Taq PCR kits are subjected to stringent quality control tests, are free of contaminating exo- and endonuclease activity, and meet strict requirements with respect to DNA contamination levels.

KAPA Taq PCR Protocol

KAPA Taq DNA Polymerase can be used to replace any commercial *Taq* DNA polymerase in an existing protocol. To allow the most seamless integration of KAPA Taq into existing protocols, be sure to match reaction conditions, particularly the $MgCl_2$, primer and enzyme concentrations, as closely as possible.

Step 1: Prepare the PCR master mix

- Ensure that all reagents are properly thawed and mixed.
- Prepare a PCR master mix containing the appropriate volume of all reaction components common to all or a subset of reactions to be performed.
- Calculate the required volumes of each component based on the following table:

Component	25 µL reaction[1]	Final conc.
PCR-grade water	Up to 25 µL	N/A
5X KAPA Taq Mg-free Buffer C or D	2.5 µL	1X
25 mM $MgCl_2$	As required[2]	≥1.5 mM
10 mM dNTP Mix	0.5 µL	0.2 mM each
10 µM Forward Primer	1.0 µL	0.4 µM
10 µM Reverse Primer	1.0 µL	0.4 µM
5 U/µL KAPA Taq DNA Polymerase[3]	0.1 µL	0.5 U
Template DNA	As required	As required[4]

[1] Reaction volumes of 10–50 µL are recommended. For volumes other than 25 µL, scale reagents proportionally.

[2] A final $MgCl_2$ concentration of 1.5 mM is sufficient for most standard applications. For assays that do not perform well with 1.5 mM $MgCl_2$, the optimal $MgCl_2$ concentration for each primer–template combination should be determined empirically.

[3] For GC-rich and other difficult templates, higher enzyme concentrations (up to 2.5 U per 25 µL reaction) may be required.

[4] ≤250 ng for genomic DNA; ≤25 ng for less complex DNA (e.g. plasmid, lambda).

NOTE: For GC-rich or other difficult templates or amplicons, include DMSO at a final concentration of 5%.

Step 2: Set up individual reactions

- Transfer the appropriate volume of PCR master mix, template and primer to individual PCR tubes/wells of a PCR plate.
- Cap or seal individual reactions, mix and centrifuge briefly.

Step 3: Run the PCR

- Perform PCR with the following cycling protocol:

Step	Temperature	Duration	Cycles
Initial denaturation	95°C	3 min[1]	1
Denaturation	95°C	30 sec	35[3]
Annealing[2]	T_m – 5°C	30 sec	
Extension	72°C	1 min/kb	
Final extension (optional)[4]	72°C	1 min/kb	1
Hold	4–10°C	∞	1

[1] Initial denaturation for 3 min at 95°C is recommended for most assays. For GC-rich targets (>65% GC content), 5 min at 95°C may be used.

[2] An annealing temperature 5°C lower than the calculated melting temperature (T_m) of the primer set is recommended as first approach. If low yields and/or nonspecific amplification is obtained, an annealing temperature gradient PCR is recommended to determine the optimal annealing temperature for the primer set empirically.

[3] 35 cycles are sufficient for most assays. A higher number of cycles may be necessary for assays requiring a higher level of sensitivity.

[4] Final extension should be included if PCR products are to be cloned into TA cloning vectors.

KAPABIOSYSTEMS

Headquarters, United States
Wilmington, Massachusetts
Tel: 781.497.2933
Fax: 781.497.2934

Manufacturing, R&D
Cape Town, South Africa
Tel: +27.21.448.8200
Fax: +27.21.448.6503

Technical Support
kapabiosystems.com/support

Sales
sales@kapabiosystems.com