KAPABIOSYSTEMS

Technical Data Sheet

KAPA Taq ReadyMix PCR Kit

KR0354_S – v3.20

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 ReadyMix (2X) is a ready-to-use cocktail containing all components for PCR, except primers and template. The 2X ReadyMix contains KAPA Taq DNA Polymerase (0.5 U per 25 µL reaction), KAPA Taq Buffer, dNTPs (0.2 mM of each dNTP at 1X), $MgCl_2$ (1.5 mM at 1X) and stabilizers.

KAPA Taq ReadyMix is available with and without dye. The 2X ReadyMix with dye contains two inert tracking dyes to enable direct loading of PCR products onto agarose gels for analysis by electrophoresis, without the need to add a DNA loading solution.

Product Applications

KAPA Taq ReadyMixes are ideally suited for:

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

KAPA Taq ReadyMix with dye is recommended when reaction products are analyzed by agarose gel electrophoresis.

Product Specifications

Shipping and Storage

KAPA Taq ReadyMix kits are shipped on dry ice or ice packs, depending on the country of destination. Upon arrival, store kit components at -15°C to -25°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 ReadyMixes contain isostabilizers and may not freeze solidly, even when stored at -15°C to -25°C. This will not affect the shelf-life of the product.

Kit Codes and Components	
KK1006 (5 x 1.25 mL)	2X KAPA Taq ReadyMix contains 1.5 mM $MgCl_2$ (1X)
KK1024 (5 x 1.25 mL)	2X KAPA Taq ReadyMix with dye contains 1.5 mM $MgCl_2$ (1X)

Quick Notes

- KAPA Taq ReadyMix can replace any commercial *Taq* DNA polymerase in an existing protocol. The annealing temperature may need to be optimized to account for differences in formulation.
- 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.
- The 2X KAPA Taq ReadyMix with dye includes two inert tracking dyes, which allow loading of PCR products directly onto agarose gels for analysis.
- KAPA Taq ReadyMixes contain 1.5 mM $MgCl_2$ and 0.2 mM of each dNTP (at 1X).

Handling

Always ensure that the product has been fully thawed and mixed before use. Reagents may be stored at 2°C to 8°C for short-term use (up to 1 month). Return to -15°C to -25°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.

Safety Information

Precautions

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material can vary, the operator must optimize pathogen inactivation and follow the appropriate measures according to local safety regulations.
- Do not eat, drink, or smoke in the laboratory area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats, and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online at www.sigmaaldrich.com, or upon request from www.sigma-aldrich.com/techservice.

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
2X KAPA Taq ReadyMix (1.5 mM $MgCl_2$ at 1X)[2]	12.5 µL	1X
10 µM Forward Primer	0.5–1.25 µL	0.2–0.5 µM
10 µM Reverse Primer	0.5–1.25 µL	0.2–0.5 µM
Template DNA[3]	As required	As required

[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] ≤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.

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