KAPABIOSYSTEMS

Technical Data Sheet

KAPA Taq HotStart PCR Kit

KR0355 – v4.16

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.

In the HotStart formulation, the enzyme is combined with a proprietary antibody that inactivates the enzyme until the first denaturation step. This prevents nonspecific amplification during reaction setup, increases sensitivity, and improves reaction efficiency. PCR products generated with KAPA Taq HotStart are A-tailed and may be cloned into TA cloning vectors.

KAPA Taq HotStart Buffer is a uniquely-formulated buffer to facilitate specific primer annealing. This translates to higher yields of specific product when compared to traditional *Taq* buffers, and improved amplification of GC- and AT-rich templates. However, KAPA Taq HotStart DNA Polymerase may be used in combination with any standard *Taq* buffer with a pH of 8.3 or higher.

Product Applications

The KAPA Taq HotStart PCR Kit is ideally suited for:

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

Product Specifications

Shipping and Storage

KAPA Taq HotStart 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 HotStart Buffer contains isostabilizers and may not freeze solidly, even when stored at -20°C. This will not affect the shelf-life of the product.

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.

Kapa/*Roche* Kit Codes and Components	
KK1508 *07958765001* (250 U) **KK1510** *07958781001* (500 U) **KK1513** *07958811001* (2500 U)	KAPA Taq HotStart DNA Polymerase (5 U/µL) KAPA Taq HotStart Buffer (5X) $MgCl_2$ (25 mM)
KK1509 *07958773001* (250 U) **KK1511** *07958790001* (500 U)	KAPA Taq HotStart DNA Polymerase (5 U/µL) KAPA Taq HotStart Buffer (5X) $MgCl_2$ (25 mM) KAPA dNTP Mix (10 mM each)
KK1512 *07958803001* (2500 U)	KAPA Taq HotStart DNA Polymerase (5 U/µL)

Quick Notes

- KAPA Taq HotStart DNA Polymerase can replace any commercial hotstart *Taq* DNA polymerase in an existing protocol. The final $MgCl_2$ concentration and annealing temperature may need to be optimized to account for differences in formulation.
- The KAPA Taq HotStart Buffer is a uniquely-formulated buffer offering improved specificity and sensitivity, and improved amplification of GC- and AT-rich templates.
- The KAPA Taq HotStart Buffer does not contain $MgCl_2$; $MgCl_2$ (25 mM) is supplied separately to allow greater flexibility during reaction setup.
- The KAPA Taq HotStart PCR Kit is suitable for the amplification of fragments up to 3.5 kb from genomic DNA or 5 kb from less complex targets.

Quality Control

Each batch of KAPA Taq HotStart DNA Polymerase is confirmed to contain <2% contaminating protein (Agilent Protein 230 Assay). KAPA Taq HotStart 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 HotStart PCR Protocol

KAPA Taq HotStart DNA Polymerase can be used to replace any commercial hotstart *Taq* DNA polymerase in an existing protocol. To allow the most seamless integration of KAPA Taq HotStart 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 HotStart Buffer	5.0 µL	1X
25 mM $MgCl_2$	1.5 µL	1.5 mM[2]
10 mM dNTP Mix	0.5 µL	0.2 mM each
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
5 U/µL KAPA Taq HotStart DNA Polymerase[3]	0.1 µL	0.5 U
Template DNA[4]	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] 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.

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