KAPABIOSYSTEMS

Technical Data Sheet

KAPA2G Robust HotStart PCR Kit

KR0380 – v6.16

Product Description

KAPA2G Robust HotStart PCR Kits contain KAPA2G Robust HotStart DNA Polymerase, a second-generation enzyme engineered through a process of directed evolution. KAPA2G Robust DNA Polymerase was engineered for improved processivity and inhibitor tolerance, and offers significantly improved performance with challenging assays when compared to wild-type *Taq* DNA polymerase. In the HotStart formulation, the enzyme is combined with a proprietary antibody that inactivates the enzyme until the first denaturation step. This eliminates spurious amplification products resulting from nonspecific priming events during reaction setup and initiation, and increases overall reaction efficiency.

The enzyme is supplied with three reaction buffers, and a proprietary enhancer for GC-rich PCR. KAPA2G Buffer A is specifically formulated for the unique characteristics of the engineered KAPA2G enzymes, while KAPA2G Buffer B is recommended when template samples are contaminated with PCR inhibitors. KAPA2G GC Buffer is recommended for GC-rich PCR, while KAPA Enhancer 1, a proprietary DNA destabilizer, may be combined with either KAPA2G Buffer A or B in GC-rich assays, or assays where secondary structure is problematic.

DNA fragments generated with KAPA2G Robust HotStart DNA Polymerase have the same characteristics as DNA fragments generated with wild-type *Taq* DNA polymerase, and may be used for routine downstream analyses or applications, including restriction enzyme digestion, cloning, and sequencing. Like wild-type *Taq*, KAPA2G Robust HotStart has 5'→3' polymerase and 5'→3' exonuclease activities, but no 3'→5' exonuclease (proofreading) activity. The fidelity of KAPA2G Robust HotStart is similar to that of wild-type *Taq*; it has an error rate of approximately 1 error per 1.7 x 10^5 nucleotides incorporated. PCR products generated by KAPA2G Robust HotStart are 3'-dA-tailed and may be cloned into TA cloning vectors.

Product Applications

KAPA2G Robust HotStart PCR Kits are ideally suited for the amplification of DNA fragments <5 kb in standard end-point PCR assays from a wide variety of templates. They are particularly suited for:

- Assays which perform poorly with wild-type *Taq*
- Amplification of DNA fragments with high GC- or AT-content
- Amplification from template samples that contain PCR inhibitors (e.g. salts, urea, SDS, ethanol, EDTA) at concentrations that inhibit wild-type *Taq*
- Amplification from crude samples, e.g. colony PCR, or PCR from crude extracts, such as those prepared using KAPA Express Extract.

Visit **www.kapabiosystems.com** for additional product information, or contact Technical Support at **kapabiosystems.com/support** for advice on the best KAPA2G Robust product for your application.

Kapa/*Roche* Kit Codes and Components	
KK5522 *07961103001* (100 U) **KK5515** *07961057001* (250 U) **KK5517** *07961073001* (500 U)	KAPA2G Robust HotStart DNA Polymerase (5 U/µL) KAPA2G Buffer A (5X) KAPA2G Buffer B (5X) KAPA2G GC Buffer (5X) KAPA Enhancer 1 (5X) KAPA $MgCl_2$ (25 mM)
KK5532 *07961197001* (100 U) **KK5516** *07961065001* (250 U) **KK5518** *07961081001* (500 U)	KAPA2G Robust HotStart DNA Polymerase (5 U/µL) KAPA2G Buffer A (5X) KAPA2G Buffer B (5X) KAPA2G GC Buffer (5X) KAPA Enhancer 1 (5X) KAPA $MgCl_2$ (25 mM) KAPA dNTP Mix (10 mM each)

Quick Notes

- KAPA2G Robust HotStart PCR Kits contain KAPA2G Robust HotStart DNA Polymerase, engineered for high processivity and inhibitor tolerance.
- Both purified genomic DNA and crude samples (e.g. colony PCR) can be used as template.
- Use 0.5 U of KAPA2G Robust HotStart DNA Polymerase per 25 µL reaction. More challenging PCRs (GC-rich, crude sample) may require higher enzyme concentrations.
- Use 15 sec/kb extension time per cycle, and increase to 30–60 sec/kb for difficult amplicons or templates.
- KAPA2G Buffers contain 1.5 mM $MgCl_2$ at 1X. Additional $MgCl_2$ may be added using the 25 mM $MgCl_2$ solution provided in the kit.
- KAPA2G Buffer A is optimized for high yield, specificity, and sensitivity.
- KAPA2G Buffer B is recommended for inhibitor-contaminated template samples.
- KAPA2G GC Buffer is recommended for GC-rich PCR (>70% GC).
- KAPA Enhancer 1 can be used with KAPA2G Buffer A or B, particularly for GC-rich assays.
- Reaction products are 3'-dA-tailed and may be cloned into TA cloning vectors.

Standard PCR Protocol

IMPORTANT! The KAPA2G Robust HotStart PCR Kit contains an engineered DNA polymerase and uniquely-formulated buffer, and requires specialized reaction conditions. If these conditions are not adhered to, reaction failure is likely. Refer to **Important Parameters** for more information.

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 KAPA2G Buffer A or 5X KAPA2G Buffer B or 5X KAPA2G GC Buffer[2]	5.0 µL	1X
5X KAPA Enhancer 1[3]	5.0 µL	1X
10 mM KAPA dNTP Mix	0.5 µL	0.2 mM each
10 µM Forward Primer	1.25 µL	0.5 µM
10 µM Reverse Primer	1.25 µL	0.5 µM
Template DNA[4]	As required	As required
5 U/µL KAPA2G Robust HotStart DNA Polymerase[5]	0.1 µL	0.5 U

[1] For volumes smaller than 25 µL, scale reagents down proportionally. Reaction volumes >25 µL are not recommended.

[2] KAPA2G Buffers contain 1.5 mM $MgCl_2$ at 1X. Reactions may be supplemented with additional $MgCl_2$ if required.

[3] KAPA Enhancer 1 is an optional additive, which may not improve results for all assays. Perform reactions with and without KAPA Enhancer 1 to determine if it is required for a specific assay.

[3] Use <100 ng genomic DNA (10–100 ng) and <1 ng less complex DNA (0.1–1 ng) per 25 µL reaction as first approach.

[4] Use 0.5 U KAPA2G Robust HotStart per 25 µL reaction. For GC-rich or other difficult targets, this may be increased to 1 U. The amount of enzyme may also be increased for crude samples, samples containing inhibitors, and the amplification of longer targets.

NOTE: For detailed instructions for GC-rich PCR with the KAPA2G Robust PCR Kit, refer to **Important Parameters: GC-rich PCR**.

Step 2: Set up individual reactions

- Transfer the appropriate volumes of PCR master mix, template and primer to individual PCR tubes or 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[1]	95°C	3 min	1
Denaturation	95°C	15 sec	30–40[4]
Annealing[2]	55–65°C	15 sec	
Extension[3]	72°C	15–60 sec/kb	
Final extension	72°C	1 min/kb	1

[1] Initial denaturation for 3 min at 95°C is sufficient for most applications. Use 5 min at 95°C for GC-rich targets (>70% GC content).

[2] Calculate primer T_m (basic) and use 55°C for primers with calculated T_m of <55°C, 60°C for primers with calculated T_m of 55–65°C, and 65°C for primers with calculated T_m above 65°C. Refer to **Important Parameters: Annealing temperature** for more information.

[3] Use 15 sec extension per cycle for targets ≤1 kb, and 30–60 sec/kb for longer fragments, or to improve yields.

[4] The number of cycles required is dependent on the size of the amplicon, and the amount of template copies per reaction. A 35-cycle PCR can typically amplify a high yield of product from 100 copies of template. For crude samples, higher cycle numbers may be required.

Important Parameters

Cycling protocol

KAPA2G Robust HotStart is a highly processive DNA polymerase, capable of amplifying DNA fragments of up to 1 kb in size with a 15 sec/cycle extension time. The use of excessive extension times is likely to result in smearing and nonspecific amplification. For amplicons <1 kb with genomic DNA as template, 15 sec per cycle should be sufficient for a high yield of product. In the case of longer amplicons, the time may be increased to a maximum of 60 sec/kb, in 15 sec increments.

Assays that are likely to require longer extension times include extremely GC-rich targets (>75% GC), as well as amplification from crude samples, or template samples that are contaminated with inhibitors. Should the initial assay with a 15 sec/kb extension time produce low yields or no product at all, the extension time may be increased to a maximum of 60 sec/kb, in 15 sec increments. Note that if amplification fails completely, annealing temperature optimization is likely to be required.

In addition to extension time, the annealing time is critical to ensure success. KAPA2G Robust HotStart has much higher activity than wild-type *Taq* at temperatures typically used for annealing (~60°C), so the use of excessive annealing times often results in the same effects as excessive extension times. Typically, the formation of nonspecific products that are larger than the target band indicates that the annealing time used is too long.

Annealing temperature

Generally, an annealing temperature of 60°C produces good results with KAPA2G Robust HotStart. Use 55°C for primers with calculated T_m of <55°C, 60°C for primers with calculated T_m of 55–65°C, and 65°C for primers with calculated T_m above 65°C. If necessary, optimize annealing temperatures with gradient PCR or adjust annealing temperatures as follows:

- If a low yield of only the specific product is obtained, lower the annealing temperature in 2°C increments.
- If nonspecific products are formed in addition to the specific product, increase the annealing temperature in 2°C increments.
- If no product is formed (specific or nonspecific), reduce the annealing temperature by 5°C. If primers are highly AT-rich, $MgCl_2$ concentration may have to be increased.
- If only nonspecific products are formed (in a ladder-like pattern), increase the annealing temperature by 5°C or try recommendations for GC-rich PCR (see **Important Parameters: GC-rich PCR**).

KAPA2G Buffers and Enhancer 1

KAPA2G Buffer A is the preferred buffer for use with the KAPA2G Robust HotStart DNA Polymerase, since it is uniquely formulated for the engineered enzyme's characteristics. Therefore, when evaluating the kit for the first time, start by using KAPA2G Buffer A, or evaluate KAPA2G Buffer A and B in parallel. KAPA2G Buffer B is recommended when template samples are contaminated with inhibitors, particularly salts, as the buffer has a lower salt content than KAPA2G Buffer A. KAPA2G GC Buffer is recommended for GC-rich PCR (refer to **Important Parameters: GC-rich PCR** for more information).

KAPA Enhancer 1 is a proprietary PCR additive (DNA destabilizer) that improves reaction efficiency and specificity for some, but not all, primer-template combinations. For problematic assays, first try KAPA2G Buffer A or B, with or without 1X KAPA Enhancer 1 before further optimization is attempted. **Do not** combine KAPA2G GC Buffer and KAPA Enhancer 1.

$MgCl_2$ concentration

KAPA2G Buffers contain 1.5 mM $MgCl_2$ (1X), which is sufficient for most applications. Amplification of longer (>2 kb) and AT-rich fragments (<40% GC), as well as amplification using primers with a low GC content (<40%), is likely to require additional $MgCl_2$.

Primers

Primers should be designed to eliminate the possibility of primer-dimer formation and nonspecific annealing, and should have a GC content of 40–60%. Primers with a GC content >60% may require higher denaturation temperatures and/or longer denaturation times, while primers with a GC content <40% may require annealing temperatures <60°C, and/or increased $MgCl_2$ and primer concentrations. Furthermore, primer sets should be designed to have similar theoretical melting temperatures.

NOTE: Always dilute and store primers in a buffered solution (e.g. 10 mM Tris-HCl, pH 8.0 – 8.5) instead of PCR-grade water to limit degradation and maintain primer quality.

GC-rich PCR

The KAPA2G Robust HotStart PCR Kit has several options for GC-rich PCR. These include using the KAPA2G GC Buffer, the KAPA2G GC Buffer + 4% DMSO, or KAPA2G Buffer A + 5% DMSO + 1X KAPA Enhancer 1. For full details, refer to the **Routine GC-rich PCR Application Note** available from **www.kapabiosystems.com**.

Product Specifications

Shipping, storage and handling

KAPA2G Robust 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. KAPA2G Buffer A contains isostabilizers and may not freeze solidly, even when stored at -20°C. This will not affect the shelf-life of the product.

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. Provided that all components have been handled carefully and not contaminated, the kit is not expected to be compromised if left (unintentionally) at room temperature for a short period of time (up to 3 days). Long-term storage at room temperature and 4°C is not recommended. Please note that reagents stored at temperatures above -20°C are more prone to degradation when contaminated during use, and therefore storage at such temperatures is at the user's own risk.

Quality control

Each batch of KAPA2G Robust HotStart DNA Polymerase is confirmed to contain <2% contaminating protein (Agilent Protein 230 Assay). KAPA2G Robust 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.

Troubleshooting

Symptoms	Possible causes	Solutions
No amplification or low yield	Cycling protocol	Increase the extension time to a maximum of 60 sec/kb (in 15 sec increments). Increase the number of cycles.
	Annealing temperature is too high	Reduce the annealing temperature by 5°C. Optimize the annealing temperature by gradient PCR.
	Template DNA quantity and quality	Excess template DNA chelates Mg^{2+}. Either reduce the template concentration to <100 ng, or increase $MgCl_2$. Check template DNA quality. Store and dilute in a buffered solution, not water. Template may contain inhibitors. Perform dilution series PCR to determine optimal template concentration.
	Primer concentration	Some primers anneal more efficiently than others. Increase the primer concentration, or optimize $MgCl_2$ to improve primer binding. Store and dilute primers in a buffered solution, not water.
	$MgCl_2$	Optimize $MgCl_2$ concentration. AT-rich PCR typically requires more $MgCl_2$.
Nonspecific amplification or smearing	Template DNA	Use <100 ng of DNA per reaction, or reduce the number of cycles. Check template DNA quality.
	Cycling protocol	Excessive annealing and/or extension times will result in nonspecific amplification, typically of bands larger than the target band. Reduce the annealing and extension times to a minimum of 10 sec each. If you are using a slow-ramping cycler (<3°C/sec heating/cooling rate), reduce the denaturation and annealing times to 10 sec each, with 10 sec extension per cycle.
	Annealing temperature is too low	A sub-optimal annealing temperature will result in nonspecific amplicons that are typically smaller than the target band. See **Important Parameters: Annealing Temperature**.
	Target GC content	Try the KAPA2G GC Buffer, with and without 4% DMSO, or KAPA2G Buffer A + 1X KAPA Enhancer 1 + 5% DMSO. Refer to the **Routine GC-rich PCR Application Note** for more information.
	Enzyme concentration	Do not exceed 1 U of KAPA2G Robust HotStart DNA Polymerase per 25 µL reaction. This results in smearing and nonspecific amplification.
	Primer concentration	Some primers anneal more efficiently than others. Decrease the primer concentration. Store and dilute primers in a buffered solution, not water.

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