KAPABIOSYSTEMS

Technical Data Sheet

KAPA2G Robust HotStart ReadyMix PCR Kit

KR0381_S – v3.20

Product Description

KAPA2G Robust is a second-generation DNA polymerase engineered through a process of directed evolution. KAPA2G Robust 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.

In the ReadyMix PCR Kit, KAPA2G Robust HotStart DNA Polymerase is supplied in a convenient 2X ReadyMix format, containing all reaction components except primers and template. The ReadyMix contains KAPA2G Robust HotStart DNA Polymerase (1 U per 25 µL reaction) in a proprietary reaction buffer containing dNTPs (0.2 mM of each dNTP at 1X), $MgCl_2$ (2 mM at 1X) and stabilizers. A ReadyMix with dye is also available – this 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.

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 ReadyMix is 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.

Kit Codes and Components	
KK5701 (1.25 mL) **KK5702** (6.25 mL)	KAPA2G Robust HotStart ReadyMix (2X) (contains 2 mM $MgCl_2$ at 1X)
KK5704 (1.25 mL) **KK5705** (6.25 mL)	KAPA2G Robust HotStart ReadyMix with dye (2X) (contains 2 mM $MgCl_2$ at 1X)

Quick Notes

- KAPA2G Robust HotStart ReadyMix PCR Kits contain an antibody-mediated hot-start formulation of KAPA2G Robust 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 15 sec/kb extension time per cycle, and increase to 30–60 sec/kb for difficult amplicons or templates.
- KAPA2G Robust HotStart ReadyMix contains 2 mM $MgCl_2$ at 1X.
- KAPA2G Robust HotStart ReadyMix with dye includes two inert tracking dyes to allow direct loading onto agarose gels.
- KAPA2G Robust HotStart ReadyMix is ideal for single-protocol PCR, i.e. amplification of fragments varying from 25–65% GC, up to 1 kb in size, using a single reaction setup and cycling protocol.
- Reaction products are 3'-dA-tailed and may be cloned into TA cloning vectors.

In addition to these applications, KAPA2G Robust HotStart ReadyMix is also suitable for single-protocol PCR, which involves the amplification of DNA fragments <1 kb in size, with GC content varying from 25–65%. The addition of DMSO for amplicons above 65% GC enables amplification up to 85% GC. This allows consolidation of assays which typically require different reaction conditions and cycling protocols when amplified with wild-type DNA polymerases. For more information, please refer to the **Single Protocol PCR Application Note**, available from www.sigmaaldrich.com.

The KAPA2G Robust HotStart ReadyMix may be combined with KAPA Express Extract for fast and efficient DNA extraction and PCR. For more information, refer to the application notes for:

- Blood PCR
- Mammalian DNA barcoding
- Fish DNA barcoding
- FFPE PCR.

Standard PCR Protocol

IMPORTANT! The KAPA2G Robust HotStart ReadyMix 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 Robust HotStart ReadyMix[2]	12.5 µL	1X
100% DMSO (optional)[3]	1.25 µL	5%
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

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

[2] KAPA2G Robust HotStart ReadyMix contains 2 mM $MgCl_2$ at 1X. Additional $MgCl_2$ may be added separately if required.

[3] For amplicons with a GC content >65%, supplement reactions with 5% DMSO.

[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.

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]	95ºC	3 min	1
Denaturation	95ºC	15 sec	30–40[4]
Annealing[2]	55–65ºC	15 sec	
Extension[3]	72ºC	15–60 sec/kb	
Final extension	72ºC	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] KAPA2G Robust HotStart ReadyMix is uniquely formulated to allow for use of a single annealing temperature for a wide range of primer lengths, GC contents and calculated melting temperatures. Use 60°C as a first approach, and adjust only if necessary.

[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.

Product Specifications

Shipping, storage and handling

KAPA2G Robust HotStart ReadyMix PCR 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. The ReadyMix contains 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.

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. 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 2°C to 8°C is not recommended. Please note that reagents stored at temperatures above -15°C to -25°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 ReadyMix 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.

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.

The number of cycles to use is dependent on the number of template copies present at the beginning of the reaction. For routine applications, 35 cycles is sufficient for a high yield of product. However, if the template DNA contains a high number of copies, cycle numbers may be reduced accordingly.

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**).

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.

$MgCl_2$ concentration

KAPA2G Robust HotStart ReadyMix contains 2 mM $MgCl_2$ at 1X, which is sufficient for most applications. Longer amplicons (>2 kb) and AT-rich PCR, as well as amplification using primers with a low GC content (<40%), are likely to require additional $MgCl_2$.

GC-rich PCR

For GC-rich amplicons (>65% GC content), supplement reactions with 5% DMSO. Should this not result in successful amplification, use the KAPA2G Robust (HotStart) PCR Kits, according to the recommendations in the **Routine GC-rich PCR Application Note** available from www.sigmaaldrich.com.

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**.
	High target GC content	Supplement reactions with 5% DMSO (amplicons with >65% GC).
	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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