KAPABIOSYSTEMS

Technical Data Sheet

KAPA2G Fast Multiplex PCR Kit

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Product Description

KAPA2G Fast is a second-generation enzyme engineered through a process of directed evolution. KAPA2G Fast DNA Polymerase was engineered for higher processivity and speed, offering significantly faster extension rates than 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, preventing nonspecific priming and amplification during reaction setup.

KAPA2G Fast Multiplex PCR Kits are designed for high-throughput, fast multiplex PCR, in which total reaction times are 20–70% shorter than those of conventional multiplex PCR assays performed with wild-type *Taq* DNA polymerase or hotstart formulations thereof. This can be achieved without sacrificing reaction performance, and does not require specialized PCR consumables or thermocyclers.

KAPA2G Fast Multiplex Mix (2X) is a ready-to-use cocktail containing all components for fast multiplex PCR, except primers and template. The 2X Multiplex Mix contains KAPA2G Fast HotStart DNA Polymerase (1 U per 25 µL reaction), KAPA2G Buffer A (1.5X at 1X), dNTPs (0.2 mM each dNTP at 1X), $MgCl_2$ (3.0 mM at 1X) and stabilizers. This formulation facilitates primer annealing and highly specific amplification of a wide range of amplicon sizes and GC contents, resulting in more even amplification of all target fragments.

DNA fragments generated with KAPA2G Fast 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 Fast has 5'→3' polymerase and 5'→3' exonuclease activities, but no 3'→5' exonuclease (proofreading) activity. The fidelity of KAPA2G Fast 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 with KAPA2G Fast are 3'-dA-tailed and may be cloned into TA cloning vectors.

Product Applications

The KAPA2G Fast Multiplex PCR Kit is ideally suited for end-point, fast multiplex PCR of multiple DNA fragments, ranging in size from 50–1500 bp. Up to 30 different primer pairs may be combined into a single assay using the protocols provided in this document. This kit is ideally suited for:

- Typing of transgenic organisms
- Amplification of microsatellites
- Typing and detection of pathogens.

Kit Codes and Components	
KK5801 (1.25 mL) **KK5802** (6.25 mL)	KAPA2G Fast Multiplex Mix (2X) (contains 3 mM $MgCl_2$ at 1X)

Quick Notes

- KAPA2G Fast Multiplex Mix contains the engineered KAPA2G Fast HotStart DNA Polymerase, for fast and efficient multiplex PCR.
- The KAPA2G Fast Multiplex Mix contains a buffer optimized for multiplex PCR, with 0.2 mM of each dNTP and 3 mM $MgCl_2$ (at 1X).
- Use 0.2 µM of each primer, and 10–100 ng of template DNA per reaction.
- Anneal at 60°C for 30 seconds.
- Perform extension for 15 sec, and increase for longer amplicons, and/or highly multiplexed reactions.

Product Specifications

Shipping, storage and handling

KAPA2G Fast Multiplex PCR Kits are shipped on dry ice or ice packs, depending on the destination country. 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 KAPA2G Fast Multiplex Mix 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. Reagent 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 the reagent has 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 Fast HotStart DNA Polymerase is confirmed to contain <2% contaminating protein (Agilent Protein 230 Assay). KAPA2G Fast Multiplex 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.

Multiplex PCR Protocol

Multiplex PCR is a challenging application that typically requires more optimization than standard, single amplicon PCR assays. The key to successful multiplex PCR is the ability to define a single set of reaction parameters (reagent concentrations and cycling parameters) that allows for all primers to their target sequences with high specificity and be extended with the same efficiency. Primer design, as well as the enzyme and buffer system, are critical factors in this challenge.

IMPORTANT! The KAPA2G Fast Multiplex Mix 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
2X KAPA2G Fast Multiplex Mix[2]	12.5 µL	1X
10 µM Forward Primers	0.5 µL each	0.2 µM[3]
10 µM Reverse Primers	0.5 µL each	0.2 µM[3]
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, as this may result in reduced reaction efficiency.

[2] KAPA2G Fast Multiplex Mix contains 3 mM $MgCl_2$ at 1X. Reactions may be supplemented with additional $MgCl_2$ if required.

[3] When first attempting multiplex PCR with this kit, use each primer at a concentration of 0.2 µM. Depending on the results obtained, primer concentrations can then be adjusted to allow for similar yields of all fragments in the multiplex.

[4] 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.

NOTE: For GC-rich multiplex PCR assays, reactions may be supplemented with 5–10% DMSO, or 0.5–1 M betaine. Refer to **Important Parameters: Amplicon size and GC content** for more information.

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	20–35[4]
Annealing[2]	60ºC	30 sec	
Extension[3]	72ºC	15–90 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] Use of the optimal annealing temperature is critical for successful multiplex PCR. Start with 60°C, and adjust up or down as required, or perform annealing temperature gradient PCR.

[3] The extension time is dependent on the level of multiplexing, as well as the amplicon size. Refer to the table below for guidelines on the recommended extension time.

15–30 sec	30–60 sec	60–90 sec
Low plex	Medium plex	High plex
Up to 5 amplicons <1 kb in size	Up to 10 amplicons <1 kb in size	Up to 10 amplicons <1.5 kb in size
Up to 10 amplicons <500 bp in size	Up to 20 amplicons <500 bp in size	Up to 30 amplicons <500 bp in size

[4] The number of cycles used should be kept to a minimum to ensure an even yield of all amplicons in the multiplex. Start with 30 cycles, and adjust as required.

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

The KAPA2G Fast Multiplex Mix is designed for fast, efficient multiplex PCR of amplicons up to 1.5 kb in size. The cycling protocols provided in this document should be used as a guideline to determine the optimal protocol for your particular assay. Using excessive annealing or extension times may result in nonspecific amplification, smearing, primer dimer formation, and uneven yields.

Multiplex PCR typically requires longer annealing times, since multiple primers have to anneal to the template. For this reason, we recommend an annealing time of 30 sec/cycle with the KAPA2G Fast Multiplex Mix.

Annealing temperature

Multiplex PCR requires the use of primers that have similar GC contents and theoretical melting temperatures, since a single annealing temperature must allow highly efficient priming and extension of all of the primers in the multiplex.

Annealing temperature gradient PCR of the multiplex assay is recommended to determine the optimal annealing temperature for the assay. Should this not be feasible, start with an annealing temperature of 60°C, and adjust as follows:

- If a low yield of only some of the amplicons in the multiplex is obtained, with others completely absent, the annealing temperature is too high. Decrease in 2°C increments.
- If all of the amplicons in the multiplex are amplified with similar yield (low or high), the annealing temperature is optimal. Adjust yields by increasing the number of cycles, or the amount of template DNA.
- If smearing is observed in addition to all of the amplicons in the multiplex, the annealing temperature is too low. Increase in 2°C increments.
- If some amplicons in the multiplex are amplified with much higher yield than others, along with smearing and nonspecific amplification, the annealing temperature is too low. Increase in 2°C increments.

$MgCl_2$ concentration

KAPA2G Fast Multiplex Mix contains 3 mM $MgCl_2$ (1X), which is sufficient for most multiplex PCR assays. Highly multiplexed assays, longer amplicons (>1.5 kb), and AT-rich assays may require additional $MgCl_2$.

Amplicon size and GC content

For highly efficient multiplex PCR, it is best to use amplicons <500 bp in size, with GC content in the range of 40–60%. It is possible to perform GC-rich multiplex PCR with the KAPA2G Fast Multiplex Mix by supplementing reactions with either 5–10% DMSO, or 0.5–1 M betaine. Should this not result in successful amplification, consider redesigning primers to target more GC-balanced regions, or perform single reactions with the KAPA2G Robust (HotStart) PCR Kit.

Primers and template DNA

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.

High quality template DNA is essential for fast PCR. Degraded, damaged, or sheared template DNA is particularly problematic when amplifying longer fragments (>1 kb).

NOTE: Always dilute and store primers and template DNA 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 quality.

Primer concentration

Not all primers anneal with the same efficiency. To ensure even amplification of each individual amplicon within a multiplex assay, it may be necessary to adjust primer concentrations to achieve similar yields. Initially, multiplex PCR should be performed with a primer concentration of 0.2 µM each. Should some amplicons be over- or under-amplified, primer concentrations may be adjusted as required. This is especially important when combining fragments with disparate sizes in a multiplex assay, where the short, easy-to-amplify primers should be supplied at a limiting concentration so that longer amplicons can also be amplified efficiently.

When adjusting primer concentrations, take care not to increase the amount of primer in the reaction too much, as this may promote primer dimer formation and nonspecific amplification.

Alternative products for multiplex PCR

Should the KAPA2G Fast Multiplex Mix prove to be unsuitable for a particular multiplex assay, the following alternatives may be evaluated:

- KAPA LongRange HotStart, for mid- to long-range multiplex PCR (>2 kb in size)
- KAPA2G Fast HotStart PCR Kits, for highly-multiplexed assays where flexibility in reaction setup is required.
- KAPA HiFi HotStart PCR Kits, for high-fidelity amplification.
- KAPA Taq HotStart PCR Kits, for routine multiplex PCR.

For assistance with optimization, or for advice on the best Kapa product for your application, contact Technical Support at sigma-aldrich.com/techservice.

Troubleshooting

Symptoms	Possible causes	Solutions
Low yield or no amplification of some or all amplicons	Cycling protocol	Increase the extension time to a maximum of 60 sec per cycle (in 15 sec increments) for amplicons <1 kb in size. For larger amplicons, increase to a maximum of 60 sec/kb (in 30 sec increments). Increase the number of cycles.
	Annealing temperature is too high	Reduce the annealing temperature in 2°C increments. Optimize the annealing temperature by gradient PCR.
	Template DNA quantity and quality	Check template DNA quality. Store and dilute in a buffered solution, not water.
	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 and highly-multiplexed assays typically require 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 time to a minimum of 20 sec, and the extension time to a minimum of 10 sec.
	Annealing temperature is too low	A sub-optimal annealing temperature will result in smearing and nonspecific amplicons that are typically smaller than the target band. Increase the annealing temperature in 2°C increments, or perform annealing temperature gradient PCR. See **Important Parameters: Annealing Temperature**.
	High target GC content	Supplement reactions with 5–10% DMSO, or 0.5–1 M betaine.
Uneven amplification	Primer concentration	Reduce primer concentration for high-yield amplicons, and increase primer concentration for low-yield amplicons. Store and dilute primers in a buffered solution, not water.
	Annealing temperature not optimal	Annealing temperature may be slightly too high or low. Test annealing temperatures +2°C and -2°C, or perform annealing temperature gradient PCR.

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