KAPABIOSYSTEMS

Technical Data Sheet

KAPA2G Fast ReadyMix PCR Kit

KR0374 – v6.16

Product Description

KAPA2G Fast DNA Polymerase is a second-generation enzyme engineered through a process of directed evolution. KAPA2G Fast was engineered for higher processivity and speed, offering significantly faster extension rates than wild-type *Taq* DNA polymerase. The enzyme is supplied with a buffer specifically formulated for the unique characteristics of the enzyme. This optimized buffer offers improved yields, specificity, and sensitivity compared to typical wild-type *Taq* buffers.

In the ReadyMix PCR Kit, KAPA2G Fast DNA Polymerase is supplied in a convenient 2X ReadyMix format, containing all reaction components except primers and template. The ReadyMix contains KAPA2G Fast DNA Polymerase (0.5 U per 25 µL reaction) in a proprietary reaction buffer containing dNTPs (0.2 mM of each dNTP at 1X), $MgCl_2$ (1.5 mM at 1X), and stabilizers. The ReadyMix also 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.

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

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.

Other available KAPA2G Fast products include:

- KAPA2G Fast PCR Kits
- KAPA2G Fast HotStart PCR Kits
- KAPA2G Fast HotStart ReadyMix
- KAPA2G Fast Multiplex Mix
- KAPA2G Fast Genotyping Mix

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

Kapa/*Roche* Kit Codes and Components	
KK5101 *07960883001* (1.25 mL) **KK5102** *07960891001* (6.25 mL) **KK5103** *07960905001* (25 mL)	KAPA2G Fast ReadyMix with dye (2X) (contains 1.5 mM $MgCl_2$ at 1X)

Quick Notes

- KAPA2G Fast ReadyMix PCR Kits contain the engineered KAPA2G Fast DNA Polymerase, developed for fast PCR.
- Use 1 sec extension time for amplicons <1 kb and 15 sec/kb for longer amplicons, and save 20–70% of total reaction time.
- No need for specialized instrumentation or PCR consumables.
- Optimized buffer system offers improved yields, specificity and sensitivity, facilitating efficient primer annealing across a wide range of primer lengths, GC contents, and melting temperatures.
- For high reaction efficiency, do not exceed 25 µL reaction volumes.
- KAPA2G Fast ReadyMix contains 1.5 mM $MgCl_2$ at 1X.
- KAPA2G Fast ReadyMix includes two inert tracking dyes to allow direct loading onto agarose gels.
- Reaction products are 3'-dA-tailed and may be cloned into TA cloning vectors.

Product Applications

Most existing PCR assays performed efficiently with wild-type *Taq* DNA polymerase may be converted to fast PCR assays with the KAPA2G Fast ReadyMix by following the protocol provided in this document.

The following assays are likely to be **unsuitable** for fast PCR with the KAPA2G Fast ReadyMix PCR Kit:

- Amplification of long fragments (>1 kb) from low target copy numbers
- Amplification of highly GC-rich fragments (>70%)
- Amplification with primers that are prone to nonspecific annealing
- Multiplex PCR
- Amplification from template samples that contain PCR inhibitors.

Although conversion to fast PCR is possible for these assay types, and other assays that do not work well with wild-type *Taq*, it is not recommended since significant optimization may be required.

For Research Use Only. Not for use in diagnostic procedures.

Standard PCR Protocol

IMPORTANT! The KAPA2G Fast 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

- Due to the high activity of the KAPA2G Fast DNA Polymerase at room temperature, reactions should be set up on ice to limit nonspecific amplification during setup.
- 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 ReadyMix[2]	12.5 µL	1X
10 µM Forward Primer	1.25 µL	0.5 µM
10 µM Reverse Primer	1.25 µL	0.5 µM
Template DNA[3]	As required	As required

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

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

[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	10–15 sec	25–40[4]
Annealing[2]	60ºC	10–15 sec	
Extension[3]	72ºC	1–15 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 Fast ReadyMix is uniquely formulated to facilitate primer annealing across a wide range of primer and amplicon lengths and GC contents. Use 60°C as a first approach, and adjust only if necessary.

[3] The denaturation, annealing, and extension times are dependent on the thermocycler ramp speed, and the yield required. Refer to the table in **Important Parameters: Cycling protocol** for guidelines on the recommended extension time.

[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.

Product Specifications

Shipping, storage and handling

KAPA2G Fast ReadyMix 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. The KAPA2G Fast ReadyMix 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 the ReadyMix 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 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 Fast DNA Polymerase is confirmed to contain <2% contaminating protein (Agilent Protein 230 Assay). KAPA2G Fast ReadyMix is subjected to stringent quality control tests, is free of contaminating exo- and endonuclease activity, and meets strict requirements with respect to DNA contamination levels.

Important Parameters

Cycling protocol

KAPA2G Fast ReadyMix is capable of amplifying fragments of up to 1 kb in size with a 1 sec/cycle extension time. Recommendations for hold times are based on the ramp rate of the thermocycler, as well as the yield required. Thermocyclers with ramp speeds higher than 3°C/sec are considered fast, while slow cyclers typically have ramp rates from 1–3°C/sec. The slower the ramp speed, the shorter the hold time required.

Standard cycling (fast cyclers) Ramp rate >3°C/sec	Maximum speed (slow cyclers) Ramp rate <3°C/sec	Maximum yield (all cyclers)
Denature 15 sec	Denature 10 sec	Denature 15 sec
Anneal 15 sec	Anneal 10 sec	Anneal 15 sec
Extend 5 sec	Extend 1 sec	Extend 15 sec

Using excessive extension times may result in nonspecific amplification, smearing, primer dimer formation, and over-amplification. If low yields are obtained even with a 15 sec extension time, increase to a maximum of 30 sec/cycle, in 5 sec increments. For amplicons >1 kb in size, start with 15 sec/kb, and increase to a maximum of 1 min/kb, in 15 sec increments.

In addition to extension time, the annealing time is critical to ensure success. At temperatures typically used for annealing (~60°C), KAPA2G Fast DNA Polymerase has much higher activity than wild-type *Taq* DNA polymerase. Therefore, 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.

$MgCl_2$ concentration

KAPA2G Buffer A contains 1.5 mM $MgCl_2$ at 1X, which is sufficient for most applications. Applications which are likely to require higher $MgCl_2$ concentrations include longer amplicons (>2 kb) and AT-rich PCR, as well as amplification using primers with a low GC content (<40%).

Amplicon size

For highly efficient fast PCR, we recommend using amplicons that are <1 kb in size, with GC content in the range of 35–65%. Longer assays can be converted to fast PCR, but may require significant assay optimization.

Annealing temperature

KAPA2G Buffer A is designed to facilitate primer annealing across a wide range of primer and amplicon lengths, and GC contents. This means that for most assays, an annealing temperature of 60°C can be used with high success rates. However, should 60°C not produce the desired result, annealing temperatures may be optimized with gradient PCR, or adjusted 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. $MgCl_2$ 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 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.

Amplification from low-complexity templates, such as plasmid DNA, generally requires minimal optimization. Applications based on low target copy numbers (e.g. when amplifying single-copy genes from genomic templates, or when using cDNA as template) are generally more challenging. For plasmid DNA, 1–10 ng template per 25 µL reaction is sufficient, whereas up to 100 ng complex genomic DNA or cDNA may be required.

GC-rich PCR

For GC-rich amplicons, supplement reactions with 5% DMSO. Should this not result in successful amplification, the KAPA2G Robust PCR Kit, which is optimized for GC-rich PCR, may be used.

Troubleshooting

Symptoms	Possible causes	Solutions
No amplification or low yield	Cycling protocol	Increase the extension time to a maximum of 30 sec per cycle (in 5 sec increments) for amplicons <1 kb in size. For larger amplicons, increase 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.
	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. Reduce the number of cycles.
	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	Supplement reactions with 5% DMSO, or try the KAPA2G Robust PCR Kit.
	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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