KAPA2G™ Fast PCR Kit

Kit components	Product codes			
	KK 5020	KK 5008	KK 5021	KK 5009
KAPA2G Fast DNA polymerase (5 U/µl)	100 U	100 U	250 U	250 U
5X KAPA2G Buffer A	1.5 ml	1.5 ml	3.0 ml	3.0 ml
$MgCl_2$ (25 mM)	1.6 ml	1.6 ml	1.6 ml	1.6 ml
dNTP Mix (10 mM each dNTP)	-	160 µl	-	300 µl

KAPA2G Buffer A contains $MgCl_2$ at a 1X concentration of 1.5 mM.

Storage, handling and specifications

Store all components at -20 °C for long-term use. Please refer to Section 6 for full details.

Quick Notes

- Save 20 – 70% in total reaction time by reducing extension times.
- Use 1 sec total extension time for amplicons <1 kb and 15 sec/kb for longer amplicons.
- Compatible with existing end-point PCR assay for single amplicons up to 5 kb.
- Optimized single-buffer system offers improved yields, specificity and sensitivity.
- Use 0.5 units KAPA2G Fast DNA Polymerase per 25 µl reaction, or less for smaller volumes.
- Do not exceed 25 µl reaction volumes.

1. Product Description

KAPA2G Fast PCR Kits contain the KAPA2G Fast DNA Polymerase, a second-generation enzyme derived through a process of molecular evolution. KAPA2G Fast DNA Polymerase was specifically engineered for higher processivity and speed, offering significantly faster extension rates than wild-type *Taq* polymerase. The enzyme is now supplied with an easy-to-use single-buffer system, specifically formulated for the unique characteristics of the enzyme. This optimized buffer offers improved yields, specificity and sensitivity across a wide range of amplicon types and lengths.

KAPA2G Fast 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* polymerase. This can be achieved without sacrificing reaction performance or the requirement for 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* 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 A-tailed and may be cloned into TA cloning vectors.

2. Applications

Any existing PCR assay performed efficiently with wild-type *Taq* polymerase may be converted to a Fast PCR assay with KAPA2G Fast DNA Polymerase. Typically, very little re-optimization of reaction parameters is required. Fast PCR assays with KAPA2G Fast may be performed with any conventional Peltier-based thermocycler and thin-walled PCR tubes or plates.

Conversion to Fast PCR is not recommended for assays that do not yield optimal results with wild-type *Taq* such as:

- Amplification of long fragments (>1 kb) from low target copy numbers.
- PCR assays involving primers that are prone to non-specific amplification (even after reaction optimization).
- Complex PCR assays, e.g. Multiplex PCRs or assays involving the incorporation of nucleotide analogs.
- Optimized assays that give low yields of the desired amplicon despite a high target copy number (e.g. amplification from difficult templates or templates containing PCR inhibitors or low sensitivity assays requiring a polymerase blend).

Although it is possible to convert such assays to Fast PCR assays, significant reaction optimization is likely to be required.

3. Reaction setup

3.1 Typical reaction setup:

A typical KAPA2G Fast reaction consists of the following:

Component	Final concentration	Volume in a 25 µl rxn
PCR grade water		Up to 25.0 µl
5X KAPA2G Buffer A	1X	5.0 µl
$MgCl_2$ (25 mM) **ONLY if final concentration >1.5 mM needed**	1.5 mM in 1X buffer	0.5 µl for each 0.5 mM $MgCl_2$ >1.5 mM
dNTP Mix (10 mM each dNTP)	0.2 mM each dNTP	0.50 µl
Forward primer (10 µM)	0.50 µM	1.25 µl
Reverse primer (10 µM)	0.50 µM	1.25 µl
DMSO (for amplicons with a GC content >60%)	5.0 – 7.5%	1.25 – 1.875 µl of a 100% solution
Template DNA	As needed	≤100 ng for genomic DNA ≤10 ng for less complex DNA (e.g. plasmid, lambda)
KAPA2G Fast DNA Polymerase (5 units/µl)	0.5 units/25 µl rxn	0.10 µl

3.2 To convert an existing PCR assay to a KAPA2G Fast assay:

- Scale reaction volume down to 25 µl or less.
- Replace the existing PCR buffer with KAPA2G Buffer A.
- Make sure that the final $MgCl_2$ concentration is the same as in the original assay.
- Use dNTPs at a final concentration of 0.2 mM each and 0.5 µM of each primer. Keep the final concentration of all other components the same as in the original assay (e.g. if DMSO is needed for the amplification of GC-rich amplicons, this should be included in the Fast reaction).
- Use 0.5 units KAPA2G Fast DNA Polymerase per 25 µl reaction, or proportionally less for smaller reaction volumes.

4. Cycling parameters

4.1 Getting started:

Standard 3-step cycling profiles with short extension times are recommended as a starting point for KAPA2G Fast assays. Because thermocyclers have different heating and cooling rates and not all PCR assays have the same reaction efficiency, recommended cycling profiles vary slightly, depending on the type of thermocycler and assay (see Table 1 on the next page). When programming your cycler for a KAPA2G Fast PCR assay, also keep the following in mind:

- Use an **initial denaturation** time of 3 min for complex genomic templates and/or the amplification of fragments >1 kb, or with a GC-content >50%. For less complex templates (e.g. plasmid or lambda DNA), the initial denaturation time may be decreased to 1 min. A denaturation time of 15 sec per cycle is sufficient for most standard, end-point assays.
- Never exceed an annealing time of 15 sec per cycle, as this may lead to non-specific amplification and/or smearing. To improve yields, rather increase the extension time (up to 15 sec/kb per cycle) or the number of cycles (from 25 – 40).
- A final extension is only needed if 3'-dA-tailing is required for fragment analysis or cloning into TA cloning vectors. In such cases, include a final extension of 1 – 10 min at 72 °C.

Table 1: Recommended KAPA2G Fast cycling profiles for different assay and thermocycler types

CYCLING STEP	STANDARD ASSAYS on SLOW RAMPING CYCLERS (≤3 °C/sec heating and cooling)	FAST RAMPING CYCLERS or GC-RICH or LONG AMPLICONS[1] (>3 °C/sec heating and cooling)
Initial denaturation	1 – 3 min at 95 °C	1 – 3 min at 95 °C
Denaturation	10 sec at 95 °C	15 sec at 95 °C
Annealing	10 sec at optimal Ta (55 – 65 °C)[2]	15 sec at optimal Ta (55 – 65 °C)[2]
Extension	1 sec at 72 °C for amplicons <1 kb 15 sec/kb at 72 °C for >1 – 5 kb amplicons	1 sec at 72 °C for amplicons <1 kb 15 sec/kb at 72 °C for >1 – 5 kb amplicons
No. of cycles	25 – 40[3] (Use same number as in original assay)	25 – 40[3] (Use same number as in original assay)
Final extension	1 – 10 min at 72 °C if products are to be TA cloned	1 – 10 min at 72 °C if products are to be TA cloned

[1] Use these parameters for standard assays on fast ramping cyclers and for GC-rich or long amplicons on fast and slow ramping cyclers
[2] For optimal results, design primers to have an optimal annealing temperature between 55 and 65 °C. Primers with lower annealing temperatures may be used, but annealing temperatures <45 °C are not recommended.
[3] The optimal number of cycles depends on template concentration. Start with 35 and increase or reduce as needed.

4.2 Further optimization:

If the recommended cycling profile yields satisfactory results, it may be possible to further reduce the cycling times for a specific assay. This can be done by systematically reducing the denaturing and/or annealing times in each cycle, or the number of cycles, up to the point where the yield of the target amplicon is not affected.

Tips:

- For fast ramping cyclers, complex targets and certain primers, longer denaturation and annealing times (but no longer than 15 sec per cycle) are needed.
- On slow ramping cyclers, the denaturation and annealing times in each cycle may be shorter (but not shorter than 10 sec).
- Touchdown assays may also be converted to Fast assays. Use the same annealing temperatures, ramping strategy and number of cycles as in the original protocol, but reduce the denaturation, annealing and extension times in each cycle to match the recommendations given in Table 1.
- For assistance with reaction setup or optimization, visit the KAPA2G Fast FAQs and other web-based technical resources on **http://www.kapabiosystems.com**, or e-mail **support@kapabiosystems.com**.

5. Troubleshooting

Only primer-dimers visible or very low yield

- Make sure reaction volumes do not exceed 25 µl.
- Increase the amount of template and/or make fresh template dilutions.
- Increase extension time in each cycle by increments of 1 sec for amplicons <1 kb and by increments of 5 sec for longer amplicons.
- Increase the number of cycles.
- Lower the annealing temperature or determine the optimal annealing temperature empirically in a gradient PCR.
- Review primer design.

Non-specific bands or high molecular weight smears

- Reduce the annealing and/or extension time in each cycle to 15 sec or less.
- Determine optimal annealing temperature empirically in a gradient PCR.
- Use a touchdown cycling protocol.
- Make fresh primer dilutions or have primers resynthesized.
- Optimize $MgCl_2$ concentration in a gradient PCR.
- Determine optimal concentration of template in a template dilution series experiment.
- Review primer design.

6. Specifications

6.1 Shipping, storage and handling

KAPA2G Fast PCR Kits are shipped on dry ice or ice packs, depending on the country of destination. Upon receipt, store the entire kit at -20 °C in a constant-temperature freezer. When stored under these conditions and handled correctly, all kit components will retain full activity until the expiry date indicated on the kit.

KAPA2G Buffer A contains isostabilizers and may not freeze solidly, even when stored at -20 °C. Nevertheless, always ensure that the 5X Buffer is fully thawed and has been vortexed before use.

KAPA2G Fast PCR Kits may be stored at 4 °C for regular, short-term use (up to 1 month). 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 short periods of time (up to 3 days). Long-term storage at room temperature or 4 °C is not recommended. Please note that reagents stored above -20 °C are more prone to degradation when contaminated by the user; storage at such temperatures is therefore at the user's own risk.

6.2 Quality control

KAPA2G Fast DNA Polymerase is extensively purified through the use of multiple chromatography steps. The final formulation contains <2% contaminating protein, as determined in an Agilent Protein 230 Assay. All batches of enzyme, buffer and other components are subjected to stringent quality control tests, are free of contaminating exo- and endonuclease activities and meet strict requirements with respect to DNA contamination.

6.3 Product use limitations and licenses

KAPA2G Fast PCR Kits are developed, designed and sold exclusively for research purposes and *in vitro* use. Neither the product, nor any individual component, was tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to the MSDS, which is available on request.

Certain applications of this product are covered by patents issued to parties other than Kapa Biosystems and applicable in certain countries. Purchase of this product does not include a license to perform any such applications. Users of this product may therefore be required to obtain a patent license depending upon the particular application and country in which the product is used.

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