



KAPA2G™ Fast ReadyMix (2X)

1. Product Description

KAPA2G Fast ReadyMix (2X) is designed for routine Fast PCR, offering significant reductions in total reaction times (20 – 70%), as well as improved performance, as compared to routine PCR performed with wild-type *Taq* DNA polymerase. This can be achieved without the requirement for specialized PCR consumables or thermocyclers.

KAPA2G Fast ReadyMix (2X) is a ready-to-use cocktail containing all components for Fast PCR, except primers and template. The 2X ReadyMix contains KAPA2G Fast DNA Polymerase (0.5 U per 25 µl reaction), KAPA2G Fast PCR Buffer, dNTPs (0.2 mM each dNTP at 1X), MgCl₂ (1.5 mM at 1X) and stabilizers. The **ReadyMix with dye** additionally contains two inert dyes, which allow for the analysis of reaction products by gel electrophoresis directly after completion of the PCR, i.e. without the need to add a DNA loading solution.

KAPA2G Fast DNA Polymerase is a second-generation enzyme derived through a process of molecular evolution. KAPA2G Fast was specifically engineered for higher processivity and speed, and is capable of significantly faster extension rates than wild-type *Taq* polymerase. The uniquely formulated KAPA2G Fast Buffer contained in the ReadyMix facilitates primer annealing and specific amplification. This allows for consistent amplification across a wide range of amplicon types, resulting in improved performance (higher yields and specificity of amplification) than those achievable with wild-type *Taq* and standard *Taq* buffers.

DNA fragments generated with KAPA2G Fast ReadyMix 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 DNA Polymerase 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 10⁵ nucleotides incorporated. PCR products generated with KAPA2G Fast ReadyMix are 3'-dA-tailed and may be cloned into TA cloning vectors.

2. Applications

KAPA2G Fast ReadyMix is designed as a convenient, high-performance replacement for wild-type *Taq* in routine, end-point PCR. It is particularly recommended for:

- Assays targeting amplicons ≤1 kb, with a GC-content ≤65%, in which purified DNA is used as the template.
- Users who wish to improve throughput (reduce cycling time) and/or improve success rates, without having to invest in specialist equipment or consumables.
- Applications employing agarose gel electrophoresis for the analysis of PCR products.

KAPA2G Fast ReadyMix with dye is not recommended for “difficult” assays, GC-rich PCR, amplification from crude samples, Multiplex PCR, amplification of long fragments, or high-fidelity PCR. To identify the best product for these applications, please visit the KAPA Biosystems website at <http://www.kapabiosystems.com>.

Kit codes and components

KK5101 100 reactions	KAPA2G Fast ReadyMix with dye (2X) 1 x 1.25 ml (100 x 25 µl rxns)
KK5102 500 reactions	KAPA2G Fast ReadyMix with dye (2X) 1 x 6.25 ml (500 x 25 µl rxns)
KK5103 2,000 reactions	KAPA2G Fast ReadyMix with dye (2X) 1 x 25 ml (2,000 x 25 µl rxns)

2X KAPA2G Fast ReadyMix with dye contains MgCl₂ 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

- KAPA2G Fast ReadyMixes contain a novel DNA Polymerase, engineered specifically for Fast PCR.
- The uniquely formulated KAPA2G Fast Buffer contained in the ReadyMix facilitates primer annealing and specific amplification.
- dNTPs and MgCl₂ are included in the ReadyMix at 1X concentrations of 0.2 mM each dNTP and 1.5 mM MgCl₂.
- 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.
- Use annealing times of 15 sec or less.
- Do not exceed 25 µl reaction volumes.
- KAPA2G Fast ReadyMix with dye allows for direct loading of PCR products into agarose gels, without the addition of a DNA loading dye solution.



3. Reaction setup

3.1 Typical reaction setup:

A typical reaction with KAPA2G Fast ReadyMix consists of the following:

Component	Final concentration	Volume in a 25 µl rxn
PCR grade water	–	Up to 25.0 µl
2X KAPA2G Fast ReadyMix	1X	12.5 µl
MgCl ₂ (25 mM) ONLY if final concentration >1.5 mM needed	1.5 mM in 1X ReadyMix	0.5 µl for each 0.5 mM MgCl ₂ >1.5 mM
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)

For reaction volumes smaller than 25 µl, scale the volumes of all components down proportionately. Reaction volumes >25 µl are not recommended.

3.2 To convert an existing PCR assay (performed with *Taq*) to a Fast PCR assay with KAPA2G Fast ReadyMix:

- If applicable: scale reaction volume down to 25 µl or less.
- Replace your existing PCR buffer, dNTPs and enzyme (or *Taq* ReadyMix) with 2X KAPA2G Fast ReadyMix.
- Make sure that the final MgCl₂ concentration is the same as in the original assay.
- Use 0.5 µM of each primer. Keep the final concentration of all other components the same as in your original assay (e.g. if DMSO is needed for the amplification of GC-rich amplicons, this should be included in the KAPA2G Fast reaction).

4. Cycling parameters

4.1 Getting started:

Standard 3-step cycling profiles with short denaturing and annealing times (10 – 15 sec per cycle) and very short extension times are recommended as a starting point for KAPA2G Fast assays. Depending on the assay setup and performance requirements (highest yield vs. shortest cycling time), the extension time may be varied between 1 sec and 15 sec/kb per cycle to obtain optimal results (see Table 1 on the next page). Although KAPA2G Fast ReadyMix may be used with any conventional, Peltier-based thermocycler, the ramp rates of the particular cyler may affect performance and optimal cycling parameters. Slow-ramping cyclers (heating and cooling at ≤1 °C/sec) typically require shorter cycling times, whereas the standard parameters – outlined in the second column of Table 1 – must be adhered to for ultra-fast ramping cyclers (>3 °C/sec heating and cooling).

When programming your cyler 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 ReadyMix cycling parameters

CYCLING STEP	STANDARD PARAMETERS (use for ultra-fast cyclers)	MAXIMUM SPEED (especially for slower cyclers)	MAXIMUM YIELD (all cyclers)
Initial denaturation	1 – 3 min at 95 °C	1 – 3 min at 95 °C	1 – 3 min at 95 °C
Denaturation	15 sec at 95 °C	10 sec at 95 °C	10 – 15 sec at 95 °C
Annealing	15 sec at optimal Ta (55 – 65 °C) ¹	10 – 15 sec at optimal Ta (55 – 65 °C) ¹	10 – 15 sec at optimal Ta (55 – 65 °C) ¹
Extension	5 sec/kb at 72 °C	1 – 5 sec/kb at 72 °C	Up to 15 sec/kb at 72 °C
No. of cycles	35 ²	25 – 35 ²	25 – 40 ²
Final extension (optional)	1 – 10 min at 72 °C	1 – 10 min at 72 °C	1 – 10 min at 72 °C

¹ 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.

² 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 with KAPA2G Fast ReadyMix. 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.

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 MgCl₂ concentration in a gradient PCR.
- Determine optimal concentration of template in a template dilution series experiment.
- Review primer design.

For advanced troubleshooting options or assistance with reaction optimization, e-mail support@kapabiosystems.com or visit <http://www.kapabiosystems.com>



KAPA2G™ Fast ReadyMix (2X)

6. Specifications

6.1 Shipping, storage and handling

KAPA2G Fast ReadyMix 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 Fast ReadyMix (2X) contains isostabilizers and may not freeze solidly, even when stored at -20 °C. Nevertheless, always ensure that the ReadyMix is fully thawed and has been vortexed before use.

KAPA2G Fast ReadyMix (2X) may be stored at 4 °C for regular, short-term use (up to 1 month). Provided that it has been handled carefully and not contaminated, the ReadyMix 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. Each batch of KAPA2G Fast ReadyMix (2X) is subjected to stringent quality control tests, is free of contaminating exo- and endonuclease activities and meets strict requirements with respect to DNA contamination.

6.3 Product use limitations and licenses

KAPA2G Fast ReadyMix 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.

For technical support please contact support@kapabiosystems.com