



KAPA2G™ Fast HotStart ReadyMix (2X)

1. Product Description

KAPA2G Fast HotStart ReadyMix Kits are designed for high throughput Fast PCR, in which total reaction times are 20 to 70% shorter than those of conventional PCR assays performed with wild-type *Taq* polymerase or hot start formulations thereof. This can be achieved without sacrificing reaction performance or the requirement for specialized PCR consumables or thermocyclers.

KAPA2G Fast HotStart ReadyMix (2X) is a ready-to-use cocktail containing all components for Fast PCR, except primers and template. The 2X ReadyMix contains KAPA2G Fast HotStart DNA Polymerase, KAPA2G Fast HotStart PCR Buffer, dNTPs (0.2 mM each dNTP at 1X), MgCl₂ (1.5 mM at 1X) and stabilizers. The **ReadyMix with dye** (KK5608 and KK5609) 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 HotStart DNA Polymerase is an antibody-mediated hot start formulation of 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. 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 non-specific priming events during reaction setup and initiation, and increases overall reaction efficiency.

DNA fragments generated with KAPA2G Fast HotStart ReadyMix have the same characteristics as those 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 HotStart has 5'-3' polymerase and 5'-3' exonuclease activities, but no 3'-5' exonuclease (proofreading) activity. The fidelity of KAPA2G Fast HotStart 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 HotStart are A-tailed and may be cloned into TA cloning vectors.

2. Applications

Any existing PCR assay performed efficiently with wild-type *Taq* polymerase (or a hot start formulation thereof) may be converted to a Fast PCR assay with KAPA2G Fast HotStart ReadyMix. Typically, very little re-optimization of reaction parameters is required. Fast PCR assays with KAPA2G Fast HotStart ReadyMix 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* or hot start formulations thereof, 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. PCRs 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.

Kit codes and components

KK5603 100 reactions	KAPA2G Fast HotStart ReadyMix (2X) 1 x 1.25 ml (100 x 25 µl rxns)
KK5601 500 reactions	KAPA2G Fast HotStart ReadyMix (2X) 1 x 6.25 ml (500 x 25 µl rxns)
KK5608 100 reactions	KAPA2G Fast HotStart ReadyMix with dye (2X) 1 x 1.25 ml (100 x 25 µl rxns)
KK5609 500 reactions	KAPA2G Fast HotStart ReadyMix with dye (2X) 1 x 6.25 ml (500 x 25 µl rxns)

KAPA2G Fast HotStart 2X ReadyMix 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 HotStart ReadyMixes contain a novel HotStart DNA Polymerase, engineered specifically for Fast PCR.
- The uniquely formulated KAPA2G Fast HotStart Buffer contained in the ReadyMix facilitates primer annealing and specific amplification.
- PCR products generated with KAPA2G Fast HotStart ReadyMix with dye can be loaded directly onto agarose gels for analysis, without the addition of DNA loading solution.
- dNTPs and MgCl₂ is 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.
- 1 min initial denaturation at 95 °C is sufficient for enzyme re-activation.
- Use annealing times of 15 sec or less.
- Do not exceed 25 µl reaction volumes.

3. Reaction setup

3.1 Typical reaction setup:

A typical reaction with KAPA Fast HotStart ReadyMix consists of the following:

	Final concentration	Volume in a 25 µl rxn
PCR grade water		Up to 25.0 µl
2X KAPA2G Fast HotStart 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 >3.0 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.

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

- Scale reaction volume down to 25 µl or less.
- Replace your existing PCR buffer, dNTPs and enzyme with 2X KAPA2G Fast HotStart 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 Fast reaction).

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 HotStart 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 cyclers for a KAPA2G Fast HotStart PCR assay, keep the following in mind:

- Use an initial denaturation/enzyme re-activation time of 1 min for standard assays. For the amplification of long or GC-rich amplicons, the initial denaturation time should be increased to 2 min.
- Since extension times are very short, reaction efficiency is dependent on sufficient annealing time. The optimal annealing time varies from one primer set and target to another. Always start with the recommended annealing time for your type of assay or thermocycler (as indicated in Table 1).
- A final extension is only needed if amplification products are to be cloned into TA cloning vectors. In such cases, use 30 sec/kb of amplicon length. If TA cloning will not be performed, the final extension step may be omitted.

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



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 are needed.
- On slow ramping cyclers, the denaturation and annealing times in each cycle may be shorter.
- Touchdown assays may also be converted to Fast assays with KAPA2G Fast HotStart 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 times recommended for the assay or cyler type 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.
- Increase the amount of enzyme to 1 U per 25 µl reaction.
- 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 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.
- Make fresh template dilutions or re-extract DNA.
- Determine optimal concentration of template in a template dilution series experiment.
- Review primer design.



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6. Specifications

6.1 Shipping, storage and handling

KAPA2G Fast HotStart ReadyMix (2X) 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 HotStart 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 HotStart 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 and its proprietary HotStart antibody are 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 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 HotStart ReadyMix (2X) 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.

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For technical support please contact support@kapabiosystems.com