



KAPA2G™ Fast HotStart

1. Product Description

KAPA2G Fast HotStart is based on 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. 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. The buffer is available either with or without MgCl₂ – KAPA2G **Buffer A** contains 1.5 mM MgCl₂ at 1X concentration, whereas KAPA2G **Buffer M** is supplied free of MgCl₂ and is specifically recommended for Multiplex PCR applications.

KAPA2G Fast HotStart is designed for high throughput 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 HotStart DNA Polymerase 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. Typically, very little re-optimization of reaction parameters is required. Fast PCR assays with KAPA2G Fast HotStart 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 components	Product codes*					
	KK 5523	KK 5530	KK 5503 5513	KK 5502 5512	KK 5501 5511	KK 5500 5510
KAPA2G Fast HotStart DNA polymerase (5 U/μl)	100 U	100 U	250 U	250 U	500 U	500 U
5X KAPA2G Buffer A or Buffer M (Mg free)	1.5 ml	1.5 ml	3.0 ml	3.0 ml	6.0 ml	6.0 ml
MgCl ₂ (25 mM)	1.6 ml	1.6 ml	1.6 ml	1.6 ml	1.6 ml	1.6 ml
dNTP mix (10 mM each)	-	160 μl	-	300 μl	-	600 μl

*Kits KK5500, 5501, 5502, 5503, 5523 and 5530 are supplied with 5X KAPA2G Buffer A, which contains MgCl₂ (1.5 mM MgCl₂ at 1X concentration).

Kits KK5510, KK5511, KK5512 and KK5513 are supplied with 5X KAPA2G Buffer M, which is identical to 5X KAPA2G Buffer A, but does not contain MgCl₂.

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.
- 1 min initial denaturation at 95 °C is sufficient for enzyme re-activation.
- Optimized single-buffer system offers improved yields, specificity and sensitivity.
- Buffer is available with MgCl₂ (Buffer A) or without (Buffer M). Buffer M is recommended for Multiplex PCR.
- Use 0.5 units KAPA2G Fast HotStart DNA Polymerase per 25 μl reaction, or less for smaller volumes.
- Do not exceed 25 μl reaction volumes.

3. Reaction setup

3.1 Typical reaction setup:

A typical KAPA2G Fast HotStart reaction consists of the following:

Component	Final concentration	25 µl
PCR grade water		Up to 25.0 µl
5X KAPA2G Buffer A <u>or</u> M	1X	5.0 µl
MgCl ₂ (25 mM)	1.5 mM MgCl ₂ in 1X Buffer A No MgCl ₂ in Buffer M	0.5 µl per 0.5 mM MgCl ₂ needed
dNTP mix (10 mM each)	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 HotStart 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 HotStart assay:

- Scale reactions down to 25 µl or less.
- Replace the existing PCR buffer with KAPA2G Buffer A or M.
- Make sure that the final MgCl₂ 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 HotStart 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 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 cyler 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.



Table 1: Recommended KAPA2G Fast HotStart 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* (>3 °C/sec heating and cooling)	MULTIPLEX PCR (fragments up to 1 kb)
Initial denaturation	1 min at 95 °C	2 min at 95 °C	2 min at 95 °C
Denaturation	10 sec at 95 °C	15 sec at 95 °C	15 sec at 95 °C
Annealing	10 sec at optimal Ta	15 sec at optimal Ta	30 sec at optimal Ta
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	10 sec at 72 °C or 15 sec at 65 °C
No. of cycles	25 – 40 (Use same number as in original assay)	25 – 40 (Use same number as in original assay)	Use same number as in original assay
Final extension	30 sec/kb at 72 °C if products are to be TA cloned	30 sec/kb at 72 °C if products are to be TA cloned	Not required

*Use these parameters for standard assays on fast ramping cyclers and for GC-rich or long amplicons on fast and slow ramping cyclers

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.

4.3 Converting existing Multiplex PCR assays to fast multiplex assays using KAPA2G Fast HotStart:

- Scale reactions down to 25 µl or less.
- Replace existing PCR buffer with **KAPA2G Buffer A at a final concentration of 1.5X** (7.5 µl buffer per 25 µl reaction). If the optimal MgCl₂ concentration for the assay is 1.5 mM, use **Mg free KAPA2G Buffer M**.
- Keep concentrations of other reagents the same, but use 1 unit of enzyme per 25 µl reaction.
- Start with the cycling profile recommended in Table 1 above.
- Optimize cycling times and individual primer concentrations if required.

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 a fresh preparation/dilution of template DNA.
- 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

- Ensure that annealing time is 15 sec or less.
- Determine optimal annealing temperature empirically in an annealing temperature gradient PCR.
- Use a touchdown cycling protocol.
- Optimize MgCl₂ concentration in a MgCl₂ gradient PCR.
- Make fresh primer dilutions or have primers resynthesized.
- Make a fresh preparation/dilution of template DNA.
- 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>

6. Specifications

6.1 Shipping, storage and handling

KAPA2G Fast HotStart 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 Buffers contain isostabilizers and may not freeze solidly, even when stored at -20 °C. Nevertheless, always ensure that the 5X Buffers are fully thawed and has been vortexed before use.

KAPA2G Fast HotStart 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 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 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