



KAPA2G™ Robust HotStart ReadyMix (2X)

1. Product Description

KAPA2G Robust HotStart ReadyMix (2X) is designed for robust, routine PCR of a wide range of targets and fragment sizes. It offers higher success rates, yields and consistency for both AT- and GC-rich amplicons. In addition, total reaction times are 20 – 50% shorter than those of conventional PCR assays performed with wild-type Taq polymerase or hot start formulations thereof.

KAPA2G Robust HotStart ReadyMix (2X) is a ready-to-use cocktail containing all components for PCR, except primers and template. The 2X ReadyMix contains KAPA2G Robust HotStart DNA Polymerase in a proprietary reaction buffer, dNTPs (0.2 mM of each dNTP at 1X), MgCl₂ (2 mM at 1X) and stabilizers.

KAPA2G Robust HotStart DNA Polymerase is an antibody-based hot start formulation of KAPA2G Robust DNA Polymerase, a second generation enzyme derived through a process of molecular evolution. KAPA2G Robust DNA Polymerase was specifically engineered for higher processivity and improved tolerance to common PCR inhibitors. The unique characteristics of the enzyme result in robust performance across a wide range of amplicon and template types, as well as faster extension rates than wild-type Taq polymerase – without compromising performance. 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.

KAPA2G Robust HotStart DNA Polymerase has 5'-3' polymerase and 5'-3' exonuclease activities, but no 3'-5' exonuclease (proofreading) activity. The fidelity of KAPA2G Robust 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. DNA fragments generated with KAPA2G Robust HotStart ReadyMix have the same characteristics as DNA fragments generated with wild-type Taq and may be used for routine downstream analyses or applications, including restriction enzyme digestion and sequencing. PCR products generated with KAPA2G Robust HotStart ReadyMix are 3'-dA-tailed and may be cloned into TA cloning vectors.

2. Applications

KAPA2G Robust HotStart ReadyMix (2X) is specifically designed for the amplification of DNA fragments ≤1 kb in length and with a range of GC contents (25 – 85% GC) using a standardized reaction setup and cycling protocol. For amplification of DNA fragments with a GC content >70%, the addition of DMSO (5%) is recommended. This allows the end-user to:

- Consolidate multiple cycling protocols developed for different amplicons into a single cycling protocol.
- Simplify workflows by replacing multiple, specialized reaction mixes based on PCR reagents from different suppliers with a single, easy-to-use ReadyMix formulation.
- Achieve more consistent results and higher success rates in 20 – 50% less cycling time.
- Significantly reduce the number of assays required for routine PCR-based screening or testing.
- Improve throughput and turnaround times.

For more information on additional applications of KAPA2G Robust HotStart ReadyMix, please refer to the KAPA2G Robust HotStart Application Notes on **Colony PCR**, **Mouse Genotyping**, **Routine GC-rich PCR** and **Single Protocol PCR**.

Kit codes and components

| | |
|--------------------------------|--|
| KK5700 Sample kit | KAPA2G Robust HotStart ReadyMix (2X) 1 x 0.25 ml (20 x 25 µl rxns) |
| KK5701 100 reactions | KAPA2G Robust HotStart ReadyMix (2X) 1 x 1.25 ml (100 x 25 µl rxns) |
| KK5702 500 reactions | KAPA2G Robust HotStart ReadyMix (2X) 1 x 6.25 ml (500 x 25 µl rxns) |

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 Robust HotStart ReadyMix contains a novel HotStart DNA Polymerase, engineered for robust PCR.
- The easy-to-use 2X ReadyMix format contains a proprietary reaction buffer, formulated for the amplification of diverse amplicons using a single PCR protocol.
- Achieve higher success rates, yields and consistency in 20 – 50% less cycling time than required for wild-type Taq.
- Amplify DNA fragments ≤1 kb with a GC content of 25 – 70% with a single reaction setup and cycling protocol.
- Add 5% DMSO for fragments with a GC content of >70%.
- Use 15 sec extension time per cycle.
- Do not exceed 15 sec annealing time per cycle.
- Annealing temperatures <55 °C are not recommended.



3. Reaction setup and cycling parameters

The standardized reaction setup recommended for KAPA2G Robust HotStart ReadyMix reactions is the following:

| Component | Final concentration | Volume in a 25 µl reaction ¹ |
|---|---------------------|---|
| PCR grade water | – | Up to 25.0 µl |
| 2X KAPA2G Robust HotStart ReadyMix (contains 2 mM MgCl ₂ at 1X) | 1X | 12.5 µl |
| Forward primer (10 µM) | 0.5 µM | 1.25 µl |
| Reverse primer (10 µM) | 0.5 µM | 1.25 µl |
| DMSO (100%) (for amplicons with a GC content >70%) | 5% | 1.25 µl |
| Template DNA ² | As needed | 10 – 100 ng for genomic DNA 1 – 10 ng for less complex DNA |

¹ For smaller reaction volumes, scale all volumes down proportionately. Do not perform reactions in a final volume >25 µl.

² Use 10 ng genomic DNA or 1 ng less complex DNA per 25 µl reaction as a starting point.

The recommended cycling protocol for KAPA2G Robust HotStart ReadyMix reactions is the following:

| Step | Temperature | Time | Number of cycles |
|-----------------------------------|-------------|-------------|------------------|
| Initial denaturation ¹ | 95 °C | 1 – 3 min | 1 |
| Denaturation ² | 95 °C | 10 – 15 sec | 35 |
| Primer annealing ² | 60 °C | 10 – 15 sec | |
| Extension ² | 72 °C | 10 – 15 sec | |
| Final extension ³ | 72 °C | 0 – 10 min | 1 |
| Cooling | 4 – 10 °C | HOLD | 1 |

¹ Use 3 min for complex genomic DNA or GC-rich templates/amplicons and 1 min for less complex templates.

² Use 15 sec with fast ramping cyclers (≥3 °C/sec) and 10 sec with slow ramping cyclers or small reaction volumes.

³ Only required if dA-tailing of PCR products is essential for fragment analysis or cloning.

For advanced troubleshooting or assistance with reaction setup or optimization, consult the KAPA2G Robust HotStart FAQs and other web-based technical resources on <http://www.kapabiosystems.com> or e-mail support@kapabiosystems.com.



4. Important parameters

4.1 Cycling parameters

The cycling protocol given in Section 3 has been validated across a wide range of amplicons ≤ 1 kb, with a GC content ranging from 25 – 85%. Although the KAPA2G Robust HotStart DNA Polymerase only needs 30 sec at 95 °C for reactivation, it is important to use sufficient denaturation times, particularly for GC-rich templates/amplicons.

Annealing time should not be increased or decreased beyond the range of 10 – 15 sec/cycle, as this may promote non-specific amplification and smearing or low reaction efficiency. To improve yields, rather increase the extension time, template concentration and/or number of cycles.

An annealing temperature of 60 °C is recommended as the starting point for routine PCR using a single, standardized protocol. However, depending on the characteristics of a particular set of amplicons to be processed together, this may be adjusted in the range of 55 °C – 65 °C. Annealing temperatures < 55 °C are not recommended.

To improve yields of problematic amplicons, the extension time may be increased to 30 sec per cycle. For subsets of “easy” amplicons, the extension time may be reduced as low as 5 sec/cycle.

4.2 Amplification of GC-rich and other problematic amplicons

It is recommended that reactions are supplemented with DMSO, to a final concentration of 5%, for the amplification of fragments with a GC content $> 70\%$. Any PCR-grade DMSO solution may be used. For particularly recalcitrant amplicons, a final DMSO concentration of up to 7.5% may be used. KAPA2G Robust HotStart ReadyMix may also be used in conjunction with 1X KAPA Enhancer 1, a proprietary PCR additive supplied in KAPA2G Robust and KAPA2G Robust HotStart PCR Kits. This has been shown to improve yields and specificity with GC-rich and other problematic amplicons.

If optimization of the template concentration, $MgCl_2$ concentration, DMSO concentration and/or annealing temperature does not yield satisfactory results with a particular primer-template combination, a KAPA2G Robust HotStart PCR Kit is recommended for further optimization. In these kits, KAPA2G Robust HotStart DNA Polymerase is supplied separately, with three proprietary KAPA2G reaction buffers and KAPA Enhancer 1, offering a wide range of optimization options.

4.3 Primers

Primer design and quality can have a significant effect on the success rates achieved in routine PCR employing a single, standardized reaction setup and cycling protocol. Primers should be carefully designed to eliminate the possibility of primer-dimer formation and spurious annealing as far as possible, and should be suitable for use with a single annealing temperature in the range of 55 – 60 °C. Always dilute and store primers in a buffered solution (e.g. 10 mM Tris-HCl, pH 8.0 – 8.5) instead of PCR-grade water. Good primer quality is particularly important for consistent and successful amplification of GC-rich fragments.

A final concentration of 0.5 μM of each primer is recommended for routine PCR using a single, standardized reaction setup. However, primer concentration may be optimized in the range of 0.1 – 1 μM for specific assays.

4.4 Template

Although KAPA2G Robust HotStart DNA Polymerase is more tolerant to common PCR inhibitors and can be used successfully with crude DNA extracts, cell lysates and certain crude samples, good quality DNA is recommended for routine PCR using a single, standardized protocol. Use 10 ng genomic DNA or 1 ng plasmid or lambda per 25 μl reaction as a first approach. To improve the yield and/or specificity of selected amplicons, the amount of template DNA may be reduced or increased. The optimal DNA concentration for smaller reaction volumes is not always directly proportional to the DNA concentration used in 25 μl reactions.

4.5 $MgCl_2$ concentration

KAPA2G Robust HotStart ReadyMix contains $MgCl_2$ at a 1X concentration of 2 mM, which has been determined to be optimal for the amplification of diverse amplicons using a standardized protocol. If more $MgCl_2$ is required for a specific primer-template combination or assay, reactions may be supplemented with any PCR-grade $MgCl_2$ solution. Add 0.5 μl of a 25 mM $MgCl_2$ solution to increase the final $MgCl_2$ concentration in a 25 μl reaction by 0.5 mM. The optimal $MgCl_2$ concentration for a specific assay may be determined empirically in a $MgCl_2$ gradient PCR.



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5. Troubleshooting

| Problem | Possible solutions |
|-----------------------------------|--|
| Non-specific amplification | <ul style="list-style-type: none">➤ Increase annealing temperature by 2 – 5 °C.➤ Decrease amount of template to a minimum of 1 ng genomic DNA/25 µl reaction.➤ Decrease annealing time to 10 sec/cycle.➤ Decrease number of cycles to 30. |
| Low yield | <ul style="list-style-type: none">➤ Decrease annealing temperature by 2 – 5 °C, but not lower than 50 °C.➤ Increase amount of template to a maximum of 100 ng genomic DNA/25 µl reaction.➤ Increase extension time to 30 sec/cycle.➤ Increase number of cycles to 40. |

6. Storage, handling and specifications

6.1 Shipping, storage and handling

KAPA2G Robust 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 for at least one year, or until the expiry date indicated on the kit.

KAPA2G Robust 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 Robust 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 Robust 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 Robust 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, has been 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.

Licensed under U.S. Patent nos. 5,338,671 and 5,587,287 and corresponding patents in other countries.

Boston, Massachusetts, United States

600 West Cummings Park, Suite 5350
Woburn, MA 01801 U.S.A.
Tel: +1 781 497 2933 Fax: +1 781 497 2934

Cape Town, South Africa

2nd Floor, Old Warehouse Building, Black River Park,
Fir Road, Observatory 7925, Cape Town, South Africa
Tel: +27 21 448 8200 Fax: +27 21 448 6503