KAPABIOSYSTEMS

Technical Data Sheet

# KAPA2G Fast HotStart PCR Kit

KR0375_S – v2.17

## Product Description

KAPA2G Fast HotStart PCR Kits contain KAPA2G Fast HotStart DNA Polymerase, a second-generation enzyme engineered through a process of directed evolution. KAPA2G Fast DNA Polymerase was engineered for higher processivity and speed, offering significantly faster extension rates than wild-type *Taq* DNA 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 nonspecific priming events during reaction setup and initiation, and increases overall reaction efficiency. The enzyme is supplied with a buffer formulated specifically for the unique characteristics of the enzyme. This optimized buffer offers improved yields, specificity, and sensitivity compared to typical wild-type *Taq* buffers.

KAPA2G Fast HotStart 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* DNA polymerase. This can be achieved without sacrificing reaction performance, and does not require 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* DNA 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 3'-dA-tailed and may be cloned into TA cloning vectors.

## Product Applications

Most existing PCR assays performed efficiently with wild-type *Taq* DNA polymerase may be converted to fast PCR assays with the KAPA2G Fast HotStart PCR Kit by following the protocol provided in this document.

The following assays are likely to be **unsuitable** for fast PCR with the KAPA2G Fast HotStart PCR Kit, and may require significant optimization:

- Amplification of long fragments (>1 kb) from low target copy numbers
- Amplification of highly GC-rich fragments (>70%)
- Amplification from template samples that contain PCR inhibitors.

| Kit Codes and Components | |
|---|---|
| **KK5523** (100 U)<br>**KK5503** (250 U)<br>**KK5501** (500 U)<br>**KK5519** (2500 U) | KAPA2G Fast HotStart DNA Polymerase (5 U/µL)<br>KAPA2G Buffer A (5X)<br>KAPA $MgCl_2$ (25 mM) |
| **KK5530** (100 U)<br>**KK5502** (250 U)<br>**KK5500** (500 U) | KAPA2G Fast HotStart DNA Polymerase (5 U/µL)<br>KAPA2G Buffer A (5X)<br>KAPA $MgCl_2$ (25 mM)<br>KAPA dNTP Mix (10 mM each) |
| **KK5513** (250 U)<br>**KK5511** (500 U) | KAPA2G Fast HotStart DNA Polymerase (5 U/µL)<br>KAPA2G Buffer M (5X)<br>KAPA $MgCl_2$ (25 mM) |
| **KK5512** (250 U)<br>**KK5510** (500 U) | KAPA2G Fast HotStart DNA Polymerase (5 U/µL)<br>KAPA2G Buffer M (5X)<br>KAPA $MgCl_2$ (25 mM)<br>KAPA dNTP Mix (10 mM each) |

### Quick Notes

- KAPA2G Fast HotStart PCR Kits contain the engineered KAPA2G Fast HotStart DNA Polymerase, developed for fast PCR.
- Use 1 sec extension time for amplicons <1 kb and 15 sec/kb for longer amplicons, and save 20–70% of total reaction time.
- No need for specialized instrumentation or PCR consumables.
- Optimized buffer system offers improved yields, specificity and sensitivity, facilitating efficient primer annealing across a wide range of primer lengths, GC contents, and melting temperatures.
- Use 0.5 U KAPA2G Fast HotStart DNA Polymerase per 25 µL reaction, or less for smaller volumes.
- For high reaction efficiency, do not exceed 25 µL reaction volumes.
- The buffer is available with and without $MgCl_2$. KAPA2G Buffer A contains 1.5 mM $MgCl_2$ at 1X, while KAPA2G Buffer M is Mg-free. Aside from $MgCl_2$ content, these two buffers are identical.
- Reaction products are 3'-dA-tailed and may be cloned into TA cloning vectors.

## Standard PCR Protocol

**IMPORTANT!** The KAPA2G Fast HotStart PCR Kit contains an engineered DNA polymerase and uniquely-formulated buffer, and requires specialized reaction conditions. If these conditions are not adhered to, reaction failure is likely. Refer to **Important Parameters** for more information.

## Step 1: Prepare the PCR master mix

- Ensure that all reagents are properly thawed and mixed.
- Prepare a PCR master mix containing the appropriate volume of all reaction components common to all or a subset of reactions to be performed.
- Calculate the required volumes of each component based on the following table:

| Component | 25 µL reaction[1] | Final conc. |
|---|---|---|
| PCR-grade water | Up to 25 µL | N/A |
| 5X KAPA2G Buffer A or M[2] | 5.0 µL | 1X |
| 10 mM KAPA dNTP Mix | 0.5 µL | 0.2 mM each |
| 10 µM Forward Primer | 1.25 µL | 0.5 µM |
| 10 µM Reverse Primer | 1.25 µL | 0.5 µM |
| Template DNA[3] | As required | As required |
| 5 U/µL KAPA2G Fast HotStart DNA Polymerase[4] | 0.1 µL | 0.5 U |

[1] For volumes smaller than 25 µL, scale reagents down proportionally. Reaction volumes >25 µL are not recommended.

[2] KAPA2G Buffer A contains 1.5 mM $MgCl_2$ at 1X, while KAPA2G Buffer M is Mg-free. Reactions may be supplemented with additional $MgCl_2$ if required.

[3] Use <100 ng genomic DNA (10–100 ng) and <1 ng less complex DNA (0.1–1 ng) per 25 µL reaction as first approach.

[4] Use 0.5 U KAPA2G Fast per 25 µL reaction. For GC-rich or other difficult targets, this may be increased to 1 U. The amount of enzyme may also be increased for crude samples, samples containing inhibitors, and the amplification of longer targets.

## Step 2: Set up individual reactions

- Transfer the appropriate volumes of PCR master mix, template and primer to individual PCR tubes or wells of a PCR plate.
- Cap or seal individual reactions, mix and centrifuge briefly.

## Step 3: Run the PCR

- Perform PCR with the following cycling protocol:

| Step | Temperature | Duration | Cycles |
|---|---|---|---|
| Initial denaturation[1] | 95ºC | 3 min | 1 |
| Denaturation | 95ºC | 15 sec | 25–40[4] |
| Annealing[2] | 60ºC | 15 sec | |
| Extension[3] | 72ºC | 1 sec or 15 sec/kb | |
| Final extension | 72ºC | 1 min/kb | 1 |

[1] Initial denaturation for 3 min at 95°C is sufficient for most applications. Use 5 min at 95°C for GC-rich targets (>70% GC content).

[2] KAPA2G Buffer A and M are uniquely formulated to facilitate primer annealing across a wide range of primer and amplicon lengths and GC contents. Use 60°C as a first approach, and adjust only if necessary.

[3] Use 1 sec extension per cycle for targets ≤1 kb, and 15 sec/kb for longer fragments, or to improve yields.

[4] The number of cycles required is dependent on the size of the amplicon, and the amount of template copies per reaction. A 35-cycle PCR can typically amplify a high yield of product from 100 copies of template.

## Product Specifications

### Shipping, storage and handling

KAPA2G Fast HotStart PCR Kits are shipped on dry ice or ice packs, depending on the country of destination. Upon arrival, store kit components at -15°C to -25°C in a constant-temperature freezer. When stored under these conditions and handled correctly, full activity of the kit is retained until the expiry date indicated on the kit label. KAPA2G Buffer A and M contain isostabilizers and may not freeze solidly, even when stored at -15°C to -25°C. This will not affect the shelf-life of the product.

Always ensure that the product has been fully thawed and mixed before use. Reagents may be stored at 2°C to 8°C for short-term use (up to 1 month). Return to -15°C to -25°C for long-term storage. 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 a short period of time (up to 3 days). Long-term storage at room temperature and 2°C to 8°C is not recommended. Please note that reagents stored at temperatures above -15°C to -25°C are more prone to degradation when contaminated during use, and therefore storage at such temperatures is at the user's own risk.

### Quality control

Each batch of KAPA2G Fast HotStart DNA Polymerase is confirmed to contain <2% contaminating protein (Agilent Protein 230 Assay). KAPA2G Fast HotStart PCR Kits are subjected to stringent quality control tests, are free of contaminating exo- and endonuclease activity, and meet strict requirements with respect to DNA contamination levels.

## Important Parameters

### Cycling protocol

KAPA2G Fast HotStart DNA Polymerase is designed to amplify fragments of up to 1 kb in size with a 1 sec/cycle extension time. Using longer extension times may result in nonspecific amplification, smearing, primer dimer formation, and over-amplification. If low yields are obtained with a 1 sec extension time, increase to a maximum of 30 sec/cycle, in 5 sec increments. For amplicons >1 kb in size, start with 15 sec/kb, and increase to a maximum of 1 min/kb, in 15 sec increments.

In addition to extension time, the annealing time is critical to ensure success. At temperatures typically used for annealing (~60°C), KAPA2G Fast HotStart DNA Polymerase has much higher activity than wild-type *Taq* DNA polymerase. Therefore, the use of excessive annealing times often results in the same effects as excessive extension times. Typically, the formation of nonspecific products that are larger than the target band indicates that the annealing time used is too long.

The number of cycles to use is dependent on the number of template copies present at the beginning of the reaction. For routine applications, 35 cycles is sufficient for a high yield of product. However, if the template DNA contains a high number of copies, cycle numbers may be reduced accordingly.

### Annealing temperature

KAPA2G Buffer A and M are designed to facilitate primer annealing across a wide range of primer and amplicon lengths, and GC contents. This means that for most assays, an annealing temperature of 60°C can be used with high success rates. However, should 60°C not produce the desired result, annealing temperatures may be optimized with gradient PCR, or adjusted as follows:

- If a low yield of only the specific product is obtained, lower the annealing temperature in 2°C increments.
- If nonspecific products are formed in addition to the specific product, increase the annealing temperature in 2°C increments.
- If no product is formed (specific or nonspecific), reduce the annealing temperature by 5°C. $MgCl_2$ concentration may have to be increased.
- If only nonspecific products are formed (in a ladder-like pattern), increase the annealing temperature by 5°C or try recommendations for GC-rich PCR (see **Important Parameters: GC-rich PCR**).

### $MgCl_2$ concentration

KAPA2G Buffer A contains 1.5 mM $MgCl_2$ at 1X, while KAPA2G Buffer M is Mg-free. A final $MgCl_2$ concentration of 1.5 mM is sufficient for most applications. PCR of longer amplicons (>2 kb) and AT-rich PCR, as well as amplification using primers with a low GC content (<40%), may require higher $MgCl_2$ concentrations.

### Primers and template DNA

Primers should be designed to eliminate the possibility of primer-dimer formation and nonspecific annealing, and should have a GC content of 40–60%. Primers with a GC content >60% may require higher denaturation temperatures and/or longer denaturation times, while primers with a GC content <40% may require annealing temperatures <60°C, and/or increased $MgCl_2$ and primer concentrations. Furthermore, primer sets should be designed to have similar theoretical melting temperatures.

High-quality template DNA is essential for fast PCR. Degraded, damaged, or sheared template DNA is particularly problematic when amplifying longer fragments (>1 kb).

**NOTE:** Always dilute and store primers and template DNA in a buffered solution (e.g. 10 mM Tris-HCl, pH 8.0–8.5) instead of PCR-grade water to limit degradation and maintain quality.

Amplification from low-complexity templates, such as plasmid DNA, generally requires minimal optimization. Applications based on low target copy numbers (e.g. when amplifying single-copy genes from genomic templates, or when using cDNA as template) are generally more challenging. For plasmid DNA, 1–10 ng template per 25 µL reaction is sufficient, whereas up to 100 ng complex genomic DNA or cDNA may be required.

### Amplicon size

For highly efficient fast PCR, we recommend using amplicons that are <1 kb in size, with GC content in the range of 35–65%. Longer assays can be converted to fast PCR, but may require significant assay optimization.

### GC-rich PCR

For GC-rich amplicons, supplement reactions with 5% DMSO. Should this not result in successful amplification, the KAPA2G Robust HotStart PCR Kit, which is optimized for GC-rich PCR, may be used.

### Thermocycler ramp speed

The protocol supplied in this kit is suitable for use on both slow and fast cycling instruments (>3°C/sec ramp speed). If your instrument has a significantly slower ramp speed, the hold times may be reduced to 10 sec each for denaturation and annealing, with a 1 sec extension time.

## Troubleshooting

| Symptoms | Possible causes | Solutions |
|---|---|---|
| No amplification or low yield | Cycling protocol | Increase the extension time to a maximum of 30 sec per cycle (in 5 sec increments) for amplicons <1 kb in size. For larger amplicons, increase to a maximum of 60 sec/kb (in 15 sec increments).<br>Increase the number of cycles. |
| | Annealing temperature is too high | Reduce the annealing temperature by 5°C.<br>Optimize the annealing temperature by gradient PCR. |
| | Template DNA quantity and quality | Excess template DNA chelates $Mg^{2+}$. Either reduce the template concentration to <100 ng, or increase $MgCl_2$.<br>Check template DNA quality. Store and dilute in a buffered solution, not water. |
| | Primer concentration | Some primers anneal more efficiently than others. Increase the primer concentration, or optimize $MgCl_2$ to improve primer binding.<br>Store and dilute primers in a buffered solution, not water. |
| | $MgCl_2$ | Optimize $MgCl_2$ concentration. AT-rich PCR typically requires more $MgCl_2$. |
| Nonspecific amplification or smearing | Template DNA | Use <100 ng of DNA per reaction, or reduce the number of cycles.<br>Check template DNA quality. |
| | Cycling protocol | Excessive annealing and/or extension times will result in nonspecific amplification, typically of bands larger than the target band. Reduce the annealing and extension times to a minimum of 10 sec each.<br>Reduce the number of cycles. |
| | Annealing temperature is too low | A sub-optimal annealing temperature will result in nonspecific amplicons that are typically smaller than the target band. See **Important Parameters: Annealing Temperature**. |
| | Target GC content | Supplement reactions with 5% DMSO, or try the KAPA2G Robust HotStart PCR Kit. |
| | Enzyme concentration | Do not exceed 1 U of KAPA2G Fast HotStart DNA Polymerase per 25 µL reaction. This results in smearing and nonspecific amplification. |
| | Primer concentration | Some primers anneal more efficiently than others. Decrease the primer concentration.<br>Store and dilute primers in a buffered solution, not water. |

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