


**KAPA2G™ Robust PCR Kit**

## 1. Product Description

KAPA2G Robust DNA Polymerase is a highly robust and versatile second-generation enzyme derived through a process of molecular evolution. The novel amino acid mutations in KAPA2G Robust offer superior performance as compared to wild-type *Taq*:

- Robust performance across a wide range of template and amplicon types and fragment sizes.
- Greatly improved tolerance to a range of common PCR inhibitors.
- Higher yield per unit of enzyme, which often translates into improved sensitivity.

The enzyme is now supplied with an improved buffer system. Buffer A is specifically formulated for the unique characteristics of the enzyme, and offers improved yield, specificity and sensitivity. KAPA Enhancer 2, previously included in KAPA2G Robust PCR Kits, has been incorporated in this buffer. Buffer B is recommended for samples containing inhibitors and for Colony PCR. The GC Buffer is specifically designed for GC-rich amplicons or templates. The proprietary additive, KAPA Enhancer 1, may be combined with Buffer A or Buffer B to improve the amplification efficiency of some, but not all, amplicons.

Like wild-type *Taq* DNA polymerase, KAPA2G Robust DNA Polymerase has 5'-3' polymerase and 5'-3' exonuclease activities, but no 3'-5' exonuclease (proofreading) activity. Using a modified *lacl* assay, the fidelity of KAPA2G Robust was confirmed to be similar to that of wild-type *Taq*; it has an error rate of approximately 1 error per  $1.7 \times 10^5$  nucleotides incorporated. Amplicons generated with KAPA2G Robust are A-tailed and are suitable for routine downstream applications, including restriction enzyme digestion, (TA) cloning and sequencing.

## 2. Applications

KAPA2G Robust PCR Kits are ideally suited for the amplification of DNA fragments up to 5 kb in standard end-point PCR assays from a wide variety of templates. It is particularly suited for:

- Amplification from templates with a high GC- or AT content.
- Templates containing common PCR inhibitors (e.g. salts, urea, SDS, ethanol or EDTA) at levels inhibitory to wild-type *Taq*.
- Amplification from crude samples, e.g. Colony PCR.
- Optimization of low yield or low specificity assays using KAPA Enhancer 1.

Kit components	Product codes			
	KK 5023	KK 5004	KK 5024	KK 5005
KAPA2G Robust DNA Polymerase (5 U/μl)	100 U	100 U	250 U	250 U
5X KAPA2G Buffer A	1.5 ml	1.5 ml	3.0 ml	3.0 ml
5X KAPA2G Buffer B	1.5 ml	1.5 ml	3.0 ml	3.0 ml
5X KAPA2G GC Buffer	1.5 ml	1.5 ml	3.0 ml	3.0 ml
5X KAPA Enhancer 1	1.5 ml	1.5 ml	3.0 ml	3.0 ml
MgCl <sub>2</sub> (25 mM)	1.6 ml	1.6 ml	1.6 ml	1.6 ml
dNTP Mix (10 mM each dNTP)	-	160 μl	-	300 μl

All 5x KAPA2G Buffers contain MgCl<sub>2</sub> 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 5 for full details.

### Quick Notes

- KAPA2G Robust DNA Polymerase may be used instead of wild-type *Taq* in most standard end-point PCR assays.
- KAPA2G Robust DNA Polymerase offers robust performance across a wide range of template and amplicon types, improved tolerance to common PCR inhibitors and higher yield/sensitivity per unit of enzyme.
- Use 30 sec/kb extension time per cycle, or 1 min/kb for difficult amplicons.
- To reduce non-specific amplification, reduce the annealing time in each cycle to 15 sec or less.
- Use 0.5 units KAPA2G Robust DNA Polymerase per 25 μl reaction. For GC-rich or other difficult amplicons, this may be increased to 1 unit per reaction.
- Use optimized Buffer A, with or without KAPA Enhancer 1, for high yields, specificity and sensitivity.
- Use Buffer B for samples containing inhibitors and Colony PCR.
- Use the GC Buffer for GC-rich amplicons and difficult templates.
- The fidelity of KAPA2G Robust DNA polymerase is the same as that of wild-type *Taq*.
- KAPA2G Robust PCR products are A-tailed and may be used for all routine downstream analyses, e.g. RE digestion and sequencing.

### 3. Reaction setup

A typical KAPA2G Robust reaction consists of the following:

Component	Final concentration	Volume in a 25 µl reaction <sup>1</sup>
PCR grade water		Up to 25.0 µl
5X KAPA2G Buffer A, B or GC Buffer <sup>2,4,5,6</sup> (contains 1.5 mM MgCl <sub>2</sub> at 1X)	1X	5.0 µl
MgCl <sub>2</sub> (25 mM) <sup>3</sup> (ONLY if final concentration >1.5 mM needed)	≥1.5 mM	0.5 µl for each 0.5 mM MgCl <sub>2</sub> >1.5 mM
5X KAPA Enhancer 1 (OPTIONAL) <sup>2,6,7</sup>	1X	5.0 µl
dNTP Mix (10 mM each dNTP)	0.2 mM each dNTP	0.50 µl
Forward primer (10 µM)	0.25 – 1.0 µM	0.25 µl for each 0.1 µM needed (e.g. 1.25 µl for 0.5 µM final)
Reverse primer (10 µM)	0.25 - 1.0 µM	0.25 µl for each 0.1 µM needed (e.g. 1.25 µl for 0.5 µM final)
Template DNA	As needed	≤100 ng for genomic DNA ≤10 ng for less complex DNA (e.g. plasmid, lambda)
KAPA2G Robust DNA Polymerase (5 units/µl) <sup>8</sup>	0.5 – 1.0 units/25 µl rxn	0.10 µl for each 0.5 U needed

#### Notes on reaction setup:

- Reaction volumes of 10 - 50 µl are recommended. For volumes larger or smaller than 25 µl, scale reagents listed in the table up or down proportionally.
- Ensure that all components are fully thawed before use. Vortex KAPA2G Buffers and KAPA Enhancer 1 before each use.
- All three 5X KAPA2G Buffers contain MgCl<sub>2</sub>. Use buffers at a final concentration of 1X (1.5 mM MgCl<sub>2</sub>). If a particular assay requires more MgCl<sub>2</sub>, supplement the reaction with the MgCl<sub>2</sub> supplied in the kit. The optimal MgCl<sub>2</sub> concentration for each application should be determined empirically in a MgCl<sub>2</sub> gradient PCR.
- KAPA2G **Buffer A** is the recommended buffer for templates or amplicons with a GC content <65%. It has been optimized for the KAPA2G Robust enzyme and offers high yields, specificity and sensitivity.
- Buffer B** has a very different composition to Buffer A and may work better for some amplicons, particularly when samples are contaminated with anionic inhibitors. It is the recommended buffer for Colony PCR. For problematic assays, first evaluate both Buffer A and Buffer B before attempting further optimization.
- KAPA2G **GC Buffer** is specifically formulated for templates or amplicons with a high GC content, or templates that are difficult to amplify as a result of stable secondary structure. For such samples, first try the GC Buffer at 1x concentration without any other additives. For particularly recalcitrant templates/amplicons, try the following:
  - 1X GC Buffer + 4% DMSO.
  - 1X Buffer A + 5% DMSO + 1X KAPA Enhancer 1.
- KAPA Enhancer 1 is a proprietary additive that improves reaction efficiency and specificity for some, but not all primer-template combinations. It is supplied as a 5X solution and should always be used at a final concentration of 1X. For problematic assays, first try Buffer A or Buffer B, with or without 1X KAPA Enhancer 1, before attempting further optimization. The GC Buffer may also be tried for problematic assays, even if the GC content of the template or amplicon is <65%. Do not combine KAPA Enhancer 1 with the GC Buffer.
- 0.5 units KAPA2G Robust DNA Polymerase per 25 µl reaction should be sufficient for most assays. For GC rich templates, double the amount of enzyme (1 unit per 25 µl reaction) is likely to improve results. The amount of enzyme may also be increased for crude samples, samples containing inhibitors and the amplification of longer amplicons. If smearing or a high background of non-specific amplicons occurs, reduce the amount of enzyme.

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



## 4. Cycling parameters

A typical KAPA2G Robust cycling profile is outlined below<sup>7,8</sup>.

Step	Temp (°C)	Time	No. of cycles
Initial denaturation <sup>1</sup>	95 °C	30 sec for low complexity templates 3 min for genomic or GC- rich DNA	1
Denaturation	95 °C	10 – 30 sec	25 – 45 (see Note 6)
Primer annealing <sup>2,9</sup>	55 – 68 °C	10 – 30 sec	
Extension <sup>3,4</sup>	72 °C	10 – 30 sec/kb (e.g. 1 min for a 2 kb amplicon)	
Final extension (OPTIONAL) <sup>5</sup>	72 °C	30 – 60 sec/kb	1
Cooling	4 – 10 °C	HOLD	1

### Notes on cycling parameters:

- For recalcitrant templates, the initial denaturation may be increased to a maximum of 10 min.
- For primers with an optimal annealing temperature (Ta) between 68 and 72 °C, a 2-step protocol with a combined annealing/extension step of 45 - 75 sec/kb at 68 – 72 °C may be used.
- 30 sec/kb extension time per cycle should be sufficient for most applications. For difficult templates or samples, this may be extended to 1 min/kb.
- For AT rich templates and amplicons, extension may be performed at 68 °C.
- A final extension is only necessary if PCR products are to be cloned into TA-cloning vectors.
- The number of cycles depends on the amount of starting material (target copy number) in the reaction. The following may be used as a general guideline:

>10 <sup>6</sup> copies	25 cycles
10 <sup>4</sup> - 10 <sup>6</sup> copies	30 cycles
<10 <sup>4</sup> copies	35 cycles

The approximate target copy number may be calculated using the formula:

$$(M \times 1,515) / bp \times (6.022 \times 10^{11}) \times P$$

where M = mass in µg of template DNA in the reaction, bp = number of base pairs of total template (not target) DNA and P = number of priming sites of primer pair on template

e.g. the target copy number for a single copy gene in 1 ng human genomic DNA equals:  $(1 \times 10^{-3}) \times 1,515 / (3.3 \times 10^9) \times (6.022 \times 10^{11}) \times 1 \approx 280$  copies

- If a very high yield of the target amplicon is obtained or if smearing or non-specific amplification occurs, try one or more of the following:
  - Reduce the annealing time to a maximum of 15 sec per cycle.
  - Reduce the extension time to 15 sec/kb.
  - Reduce the number of cycles.
  - Optimize the Ta for the specific template-primer combination in a Ta gradient PCR.
- For amplification from crude samples, e.g. Colony PCR, use 5 min initial denaturation (95 °C) and 30 sec denaturation per cycle. 15 sec annealing per cycle should be sufficient in most cases. The optimal extension rate will depend on the nature of the sample and assay.
- When designing primers, the theoretical melting temperature (Tm) of primers used together should be matched as closely as possible. As a first approach, use an annealing temperature (Ta) 3 – 5 °C lower than the lowest Tm of the two primers. For best performance, the optimal Ta for a primer pair should be determined empirically by Ta gradient PCR. Because primer melting characteristics are affected by the chemical environment, the optimal Ta for a specific primer pair should be determined in the PCR buffer used for the assay and may differ from one buffer system to another. Sample composition may also affect primer annealing, particularly if high levels of inhibitors are present. For optimal results with KAPA2G Robust, primers with annealing temperatures <55 °C are not recommended.



# KAPA2G™ Robust PCR Kit

## 5. Specifications

### 5.1 Shipping, storage and handling

KAPA2G Robust PCR Kits are shipped on 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 have been vortexed before use.

KAPA2G Robust 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.

### 5.2 Quality control

KAPA2G Robust 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. All batches 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.

### 5.3 Product use limitations and licenses

KAPA2G Robust 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](mailto:support@kapabiosystems.com)