KAPA2G™ Robust HotStart

Kit components*	Product codes		
	KK 5514 5522	KK 5515 5516	KK 5517 5518
KAPA2G Robust HotStart DNA Polymerase (5 U/µl)	100 U	250 U	500 U
5x KAPA2G Robust HotStart Buffer A (with $MgCl_2$)	1.5 ml	3.0 ml	6.0 ml
5x KAPA2G Robust HotStart Buffer B (with $MgCl_2$)	1.5 ml	3.0 ml	6.0 ml
5x KAPA2G Robust HotStart GC Buffer (with $MgCl_2$)	1.5 ml	3.0 ml	6.0 ml
5x KAPAEnhancer 1	1.5 ml	3.0 ml	6.0 ml
$MgCl_2$ (25 mM)	1.6 ml	1.6 ml	1.6 ml
dNTP mix (10 mM each)	-	300 µl (KK5516 only)	600 µl (KK5518 only)

*For the composition of larger kits, please refer to our website.

Storage

Store all components at –20 °C.

Quick Notes

- KAPA2G Robust HotStart 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.
- Use 0.5 units KAPA2G Robust HotStart DNA Polymerase per 25 µl reaction, or 1 unit per 25 µl reaction for GC-rich or other difficult templates.
- KAPA2G Robust HotStart GC Buffer is specifically formulated for GC-rich amplicons and templates.
- For other amplicons, determine the best combination of Buffer A or Buffer B, with or without KAPAEnhancer 1 for your assay.
- 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. cloning, RE digestion and sequencing.

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 DNA Polymerase offer superior performance compared to that of wild-type Taq:

- Robust performance across a wide range of templates, 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.

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 Buffers A and B and the proprietary additive, KAPAEnhancer 1, offer extended optimization options for diverse and difficult templates. KAPA2G Robust HotStart kits also contain KAPA2G Robust HotStart GC Buffer, a novel buffer formulated specifically for GC-rich templates and amplicons.

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 10^5 nucleotides incorporated.

DNA fragments generated with KAPA2G Robust HotStart have the same characteristics as DNA fragments generated with wild-type Taq polymerase and may be used for routine downstream analyses or applications, including restriction enzyme digestion and sequencing. PCR products generated with KAPA2G Robust HotStart are A-tailed and may be cloned into TA cloning vectors.

2. Applications

KAPA2G Robust HotStart kits are ideally suited for the amplification of DNA fragments up to 5 kb in standard end-point PCR assays from a 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 or ethanol) at levels inhibitory to wild-type Taq.
- Amplification from crude samples, e.g. Colony PCR.
- Optimization of low yield or low specificity assays.

3. Reaction setup

A typical KAPA2G Robust HotStart reaction consists of the following:

Component	Final concentration	Volume in a 25 µl reaction[1]
PCR grade water		Up to 25.0 µl
5x KAPA2G Robust HotStart Buffer A, B or GC Buffer[2,4,5] **(contains 1.5 mM $MgCl_2$ at 1x)**	1x	5.0 µl
$MgCl_2$ (25 mM)[3] **(ONLY if final concentration >1.5 mM needed)**	≥1.5 mM	0.5 µl for each 0.5 mM $MgCl_2$ >1.5 mM
5x KAPAEnhancer 1 **(OPTIONAL)**[6]	1x	5.0 µl
dNTP mix (10 mM each)	0.2 mM each dNTP	0.50 µl
Forward primer (10 µM)	0.1 - 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)	0.1 - 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	≤250 ng for genomic DNA ≤25 ng for less complex DNA (e.g. plasmid, lambda)
KAPA2G Robust HotStart DNA Polymerase[7] (5 units/µl)	0.5 - 1.0 units/25 µl rxn	0.10 µl for each 0.5 U needed

Notes on reaction setup:

1. Reaction volumes of 10 - 50 µl are recommended. For volumes larger or smaller than 25 µl, scale reagents listed in the above table up or down proportionally.
2. Ensure that all components are fully thawed before use. Vortex KAPA2G Robust HotStart buffers and KAPAEnhancer 1 before each use.
3. All 5x KAPA2G Robust HotStart buffers contain $MgCl_2$. Use buffers at a final concentration of 1x (1.5 mM $MgCl_2$). If a particular assay requires more $MgCl_2$, supplement the reaction with the $MgCl_2$ supplied in the kit.
4. KAPA2G Robust HotStart **Buffer A** is the recommended buffer for templates or amplicons with a GC content <65%. **Buffer B** has a very different composition and may work better for some amplicons, particularly when samples are contaminated with anionic inhibitors. For problematic assays, first evaluate both buffers before further optimization is attempted.
5. 5x KAPA2G Robust HotStart **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 KAPAEnhancer 1.
6. KAPAEnhancer 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 B, with or without 1x KAPAEnhancer 1 before further optimization is attempted. 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 the GC buffer and KAPAEnhancer 1.
7. 0.5 units KAPA2G Robust HotStart 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 HotStart FAQs and other web-based technical resources on **http://www.kapabiosystems.com** or e-mail **support@kapabiosystems.com**.

4. Cycling parameters

A typical KAPA2G Robust HotStart cycling profile is outlined below[7,8].

Step	Temp (°C)	Time	No. of cycles
Initial denaturation[1]	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[2,9]	45 - 68°C	10 - 30 sec	
Extension[3,4]	72°C	30 sec/kb *(e.g. 1 min for a 2 kb amplicon)*	
Final extension (OPTIONAL)[5]	72°C	30 - 60 sec/kb	1
Cooling	4 - 10°C	HOLD	1

Notes on cycling parameters:

1. KAPA2G Robust HotStart enzyme is fully re-activated within 30 sec, but longer initial denaturation times are required to fully denature complex or GC-rich templates. For recalcitrant templates, the initial denaturation may be increased to a maximum of 10 min.
2. 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.
3. 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.
4. For AT rich templates and amplicons, extension may be performed at 68°C.
5. A final extension is only necessary if PCR products are to be cloned into TA-cloning vectors.
6. 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:

$>10^6$ copies	25 cycles
10^4 - 10^6 copies	30 cycles
$<10^4$ 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

7. If a very high yield of the target amplicon is obtained or if smearing 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.
8. For amplification from crude samples, e.g. Colony PCR, use 5 min initial denaturation (95°C) and 30 sec denaturation and annealing time per cycle. The optimal extension rate will depend on the nature of the sample and assay.
9. 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.

5. Specifications

5.1 Shipping and storage

KAPA2G Robust HotStart kits are shipped on dry ice or ice packs, depending on the country of destination. Upon receipt, store the entire kit at -20ºC 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.

5.2 Handling

Always ensure that all kit components are fully thawed before use. Vortex 5x KAPA2G Robust HotStart buffers and KAPAEnhancer 1 after each freeze-thaw cycle. Return components to -20ºC for long-term storage.

5.3 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.

5.4 Product use limitations and licenses

KAPA2G Robust HotStart 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.

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.

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