KAPA HiFi™ HotStart

Kit components	Product codes	
	KK 2501	KK 2502
KAPA HiFi HotStart DNA Polymerase (1 U/µl)	100 U	250 U
5X KAPA HiFi Fidelity Buffer	1.5 ml	2 x 1.5 ml
5X KAPA HiFi GC Buffer	1.5 ml	2 x 1.5 ml
$MgCl_2$ (25 mM)	1.6 ml	1.6 ml
dNTP Mix (10 mM each dNTP)	160 µl	2 x 300 µl

Storage, handling and specifications

Store all components at -20 °C for long-term use. Please refer to Section 6 for full details.

Quick Notes

- KAPA HiFi HotStart PCR Kits contain a novel HotStart DNA Polymerase, engineered for fast and versatile high-fidelity PCR.
- Amplify fragments up to 15 kb from genomic DNA or 18 kb from less complex targets.
- Denature at 98 °C for 20 sec in each cycle.
- Optimal annealing temperatures are higher than in other PCR buffers, and are typically in the range of 60 – 72 °C.
- Use 15 sec extension time per cycle for amplicons ≤1 kb and 30 – 60 sec/kb per cycle for longer amplicons or to improve yields.
- Use the GC Buffer for GC-rich amplicons.
- For optimal fidelity, always use good quality DNA and the lowest number of cycles.

1. Product Description

KAPA HiFi HotStart PCR Kits are designed for routine, high-fidelity PCR of a wide range of targets and fragment sizes. It offers error rates 100X lower than that of wild-type *Taq*, as well as higher success rates, yields and consistency than those achievable with wild-type B-family (proofreading) DNA polymerases. In addition, KAPA HiFi HotStart reaction times are significantly shorter than those required for wild-type B-family DNA polymerases or hot start formulations thereof.

KAPA HiFi HotStart DNA Polymerase is an antibody-based hot start formulation of KAPA HiFi DNA Polymerase – a novel B-family DNA polymerase that exhibits industry-leading performance when compared with other high-fidelity (B-family) DNA Polymerases (i.e. single-enzyme systems) or with polymerase blends. KAPA HiFi DNA Polymerase was engineered to have increased affinity for DNA, without the need for accessory proteins or domains. The intrinsic high processivity of the enzyme results in significant improvements in yield, sensitivity, speed, as well as the ability to amplify difficult (GC- and AT-rich) and long amplicons. 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.

KAPA HiFi HotStart is supplied with two uniquely formulated PCR Buffers for optimal performance. The Fidelity Buffer is recommended for routine high-fidelity PCR (i.e. when the lowest error rates are required), whereas the GC Buffer is specifically formulated for GC-rich or difficult targets.

KAPA HiFi HotStart DNA Polymerase has 5′-3′ polymerase and 3′-5′ exonuclease (proofreading) activities, but no 5′-3′ exonuclease activity. The strong 3′-5′ exonuclease activity results in superior accuracy during DNA amplification. KAPA HiFi HotStart DNA Polymerase boasts the lowest published error rate of all B-family DNA polymerases (1 error per 2.8 x 10^7 nucleotides incorporated). This fidelity is approximately 100X higher than that of wild-type *Taq* and up to 10X higher than that of other B-family DNA polymerases and polymerase blends. DNA fragments generated with KAPA HiFi HotStart may be used for routine downstream analyses or applications, including restriction enzyme digestion and sequencing. PCR products generated with KAPA HiFi HotStart are blunt-ended, but may be 3′-dA-tailed for cloning into TA cloning vectors.

2. Applications

KAPA HiFi HotStart PCR Kits are ideally suited for routine, high-fidelity PCR including:

- Amplification of long DNA fragments for conventional sequencing (direct sequencing or sequencing of cloned PCR products).
- Amplification of short AT- and GC-rich DNA fragments for next-generation sequencing (e.g. target enrichment for resequencing).
- Amplification of DNA fragments up to 15 kb from genomic DNA or up to 18 kb from less complex targets (e.g. plasmid or lambda DNA), for cloning and protein expression or genomic characterization.
- Introduction of single or multiple point mutations using site-directed mutagenesis.

For more information on these and other high-fidelity PCR applications, please refer to the KAPA HiFi Application Notes on **Site-Directed Mutagenesis**, **Routine High-Fidelity PCR** and **High-Fidelity GC-rich PCR**.

3. Reaction setup and cycling parameters

The following reaction setup is recommended for KAPA HiFi HotStart reactions:

Component	Final concentration	Volume in a 25 μl reaction[1]
PCR grade water	–	Up to 25.0 μl
5X KAPA HiFi Fidelity or GC Buffer[2] **(contains 2.0 mM Mg^{2+} at 1X)**	1X	5.0 μl
KAPA dNTP Mix (10 mM each dNTP)[3]	0.3 mM each dNTP	0.75 μl
Forward primer (10 μM)	0.3 μM	0.75 μl
Reverse primer (10 μM)	0.3 μM	0.75 μl
DMSO (100%) **(for amplicons with a GC content >70%)**	5%	1.25 μl
Template DNA[4]	As needed	10 – 100 ng for genomic DNA 1 – 10 ng for less complex DNA
KAPA HiFi HotStart DNA Polymerase (1 U/μl)	0.5 U/25 μl rxn	0.50 μl

[1] For smaller reaction volumes, scale all volumes down proportionately. Reaction volumes >25 μl are not recommended.
[2] Use the Fidelity Buffer for most reactions. The GC Buffer is specifically formulated for amplicons/templates with a high GC content and/or stable secondary structure, and is recommended when the Fidelity Buffer produces a low yield. The additives in the GC Buffer results in a two-fold decrease in fidelity (as compared to the Fidelity Buffer).
[3] The performance of all high-fidelity DNA Polymerases, including KAPA HiFi HotStart, is highly dependent on the quality of dNTPs used; the presence of even small amounts of dUTP has a dramatic impact. Only use the highest quality KAPA dNTP Mix supplied in your kit.
[4] Use 10 ng genomic DNA or 1 ng less complex DNA per 25 μl reaction as a starting point.

The recommended KAPA HiFi HotStart cycling protocol is as follows:

Step	Temperature	Time	Number of cycles
Initial denaturation[1]	95 °C	2 – 5 min	1
Denaturation[1]	**98 °C**	**20 sec**	15 - 35[4]
Primer annealing[2]	60 – 75 °C	15 sec	
Extension[3]	72 °C	15 – 60 sec/kb	
Final extension	72 °C	1 – 5 min	1
Cooling	4 – 10 °C	HOLD	1

[1] KAPA HiFi Buffers have a higher salt concentration than conventional PCR buffers. Since buffer composition affects DNA melting, it is important to denature complex templates and targets with a high GC content sufficiently. **The above denaturation parameters must therefore be strictly adhered to.**

[2] Due to the high salt concentration of KAPA HiFi Buffers, the optimal annealing temperature for a specific primer pair is likely to be different than when used in a conventional PCR buffer. An annealing temperature of 65 °C is recommended as a starting point. Two-step cycling protocols, with a combined annealing/extension temperature in the range of 68 – 75 °C and a combined annealing/extension time of 30 sec/kb may also be used.

[3] Use 15 sec per cycle for amplicons ≤1 kb and 30 – 60 sec/kb per cycle for long amplicons or to improve yields.

[4] ≤25 cycles are recommended for most high-fidelity applications. In cases where very low template concentrations or low reaction efficiency results in low yields, 30 or 35 cycles may be performed to produce sufficient product for downstream analysis or cloning.

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

4. Important parameters

4.1 Denaturation

Due to the higher salt concentration of KAPA HiFi Buffers, it is important to use the correct denaturation parameters for KAPA HiFi HotStart reactions. Although the HotStart enzyme only requires 30 sec for activation, an initial denaturation time of 2 – 5 min at 95 °C is required to ensure that template DNA is fully denatured before the first primer annealing step. Use 5 min for complex, genomic DNA and GC-rich targets and at least 2 min for less complex templates. In each cycle, denaturation must be performed for 20 sec at 98 °C. Initial denaturation may also be performed at 98 °C, but this may lead to template damage and is not recommended for high-fidelity amplification, especially of long amplicons.

4.2 Primers and annealing temperature

Primer design and quality is important for successful DNA amplification using KAPA HiFi HotStart. Primers should be carefully designed to eliminate the possibility for primer-dimer formation and spurious annealing as far as possible and should have a GC content of 40 – 60%. Primers with a GC content >60% may require a higher denaturation temperature or longer denaturation time. Primers to be used together should have similar theoretical melting temperatures (Tm).

The actual Tm of a given primer may be affected by specific reaction conditions, including the composition of the PCR buffer, DNA concentration, presence of denaturing agents (e.g. DMSO) and nucleotide modifications (e.g. biotin or fluorescent dyes). When using KAPA HiFi HotStart for the first time with a specific primer-template combination, it is recommended that the optimal annealing temperature for the assay be determined empirically in an annealing temperature gradient PCR.

Alternatively, start with an annealing temperature of 65 °C as a first approach. To improve **sensitivity (yields)**, **reduce** the annealing temperature in increments of 1 °C (but not lower than 60 °C). To to improve **specificity**, **increase** the annealing temperature in increments of 1 °C (up to 75 °C). For long primers and primer sets with an actual optimal annealing temperature in the range of 68 – 75 °C, use a 2-step cycling protocol with a combined annealing/extension time of 30 sec/kb per cycle.

Always dilute and store primers in a buffered solution (e.g. TE or 10 mM Tris-HCl, pH 8.0 – 8.5) instead of PCR grade water.

4.3 Template DNA

Amplification from low complexity templates, such as lambda or plasmid DNA, is usually easy and should require little 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 or phage DNA, 1 – 10 ng template per 25 μl reaction is adequate, whereas up to 100 ng complex genomic DNA or cDNA may be required.

The quality of template DNA may have a significant impact on the outcome of KAPA HiFi HotStart reactions. Degraded, damaged or sheared template DNA is particularly problematic when amplifying targets >1 kb. Always dilute and store DNA in a buffered solution (e.g. TE or Tris-HCl, pH 8.0 – 8.5) instead of PCR grade water, and minimize freeze-thaw cycles to limit degradation and maintain quality. High quality template DNA is essential for high-fidelity amplification.

4.4 Amplicon length

KAPA HiFi HotStart is recommended for the amplification of fragments up to 15 kb from complex, genomic DNA and fragments up to 18 kb from less complex templates (e.g. plasmid or lambda DNA). For the efficient amplification of fragments ≥10 kb, higher template concentrations and careful optimization of the final Mg^{2+} concentration (see 4.6) may be required.

4.5 Amplification of GC-rich and other problematic amplicons

The GC Buffer is recommended for the amplification of GC-rich or other problematic amplicons. If this does not yield the desired results, try the Fidelity Buffer + 5% DMSO. The GC Buffer or Fidelity Buffer may also be used in combination with 1X KAPA Enhancer 1 (a proprietary PCR additive supplied in KAPA2G Robust and Robust HotStart PCR Kits), or with PCR grade betaine (at a final concentration of 1 M), to improve yields and/or specificity in difficult assays.

If the addition of DMSO, KAPA Enhancer 1 or betaine does not yield satisfactory results, template concentration, Mg^{2+} concentration, annealing temperature and/or extension time may have to be carefully optimized.

4.6 Mg^{2+} concentration

KAPA HiFi Buffers contain Mg^{2+} at a 1X concentration of 2.0 mM, which has been determined to be optimal for most applications. Specific applications, particularly those involving the amplification of fragments >10 kb from complex genomic templates, may require careful optimization of the final Mg^{2+} concentration. KAPA HiFi HotStart reactions may be supplemented with the 25 mM $MgCl_2$ solution supplied in the kit. Add 0.25 μl of a 25 mM $MgCl_2$ solution to increase the final Mg^{2+} concentration in a 25 μl reaction by 0.25 mM. The optimal Mg^{2+} concentration for a specific assay may be determined empirically in a $MgCl_2$ gradient PCR.

4.7 TA cloning

DNA fragments generated with KAPA HiFi HotStart may be used directly for blunt-end cloning, or cloning using restriction endonucleases. For TA cloning, 3′-dA-overhangs may be added to blunt-ended DNA fragments in a simple reaction (5 min incubation at 72 °C) containing KAPA Taq DNA Polymerase, 1X KAPA Taq Buffer and dATP (0.2 mM). It is very important to purify KAPA HiFi HotStart PCR products prior to dA-tailing. If this is not done, the 3′–5′ exonuclease activity of residual KAPA HiFi HotStart DNA Polymerase will degrade the newly generated dA-overhangs and yield a mixture of dA-tailed and blunt-ended fragments, which is likely to reduce the efficiency of TA cloning.

5. Troubleshooting

Problem	Possible solutions
Non-specific amplification or smearing	➢ Increase the annealing temperature by 2 – 5 °C. ➢ If an annealing time >15 sec per cycle was used, reduce this to 15 sec. ➢ Use less template DNA (but no less than 1 ng genomic DNA/25 µl reaction). ➢ Decrease the extension time to 15 sec/kb per cycle. ➢ Decrease the number of cycles. ➢ Prepare new, good quality template DNA and/or fresh primer stocks.
Low yield	➢ Decrease the annealing temperature by 2 – 5 °C, but not lower than 60 °C. ➢ Use more template (but no more than 100 ng genomic DNA/25 µl reaction.) ➢ Increase the extension time to 30 – 60 sec/kb per cycle and/or the number of cycles. ➢ Use the GC Buffer instead of the Fidelity Buffer. ➢ Optimize the final Mg^{2+} concentration in the reaction.

6. Storage, handling and specifications

6.1 Shipping, storage and handling

KAPA HiFi 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.

KAPA HiFi 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.

KAPA HiFi 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

KAPA HiFi 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. 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.

6.3 Product use limitations and licenses

KAPA HiFi HotStart 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

Email: info@kapabiosystems.com

KAPABIOSYSTEMS
www.kapabiosystems.com