KAPABIOSYSTEMS

Technical Data Sheet

KAPA HiFi HotStart PCR Kit

KR0369 – v9.17

Product Description

KAPA HiFi HotStart DNA Polymerase is a novel B-family DNA polymerase, engineered to have increased affinity for DNA, without the need for accessory proteins or DNA binding domains. The intrinsic high processivity of the enzyme results in significant improvement in yield, speed and sensitivity when compared to wild-type B-family DNA polymerases. In addition, the ability to amplify long targets, as well as GC- and AT-rich targets, is significantly improved.

KAPA HiFi HotStart PCR Kits are designed for routine, high-fidelity PCR of a wide range of targets and fragment sizes. They offer error rates approximately 100 times lower than wild-type *Taq* DNA polymerase, and higher success rates and yields than achievable with wild-type B-family (proofreading) DNA polymerases. In addition, KAPA HiFi requires significantly shorter reaction times than wild-type B-family DNA polymerases.

KAPA HiFi HotStart DNA Polymerase has 5'→3' polymerase and 3'→5' exonuclease (proofreading) activity, but no 5'→3' exonuclease activity. The strong 3'→5' exonuclease activity results in superior accuracy during DNA amplification, lending to KAPA HiFi HotStart DNA Polymerase the lowest published error rate of all B-family DNA polymerases (1 error per 3.6 x 10^6 nucleotides incorporated). This fidelity is approximately 100 times higher than that of wild-type *Taq* DNA polymerase, and up to 10 times higher than that of other B-family DNA polymerases and polymerase blends.

DNA fragments generated with KAPA HiFi HotStart DNA Polymerase may be used for routine downstream analysis and applications, including restriction enzyme digestion, cloning and sequencing. PCR products generated with KAPA HiFi HotStart PCR Kits are blunt-ended, but may be 3'-dA-tailed for cloning into TA cloning vectors (see **Important Parameters: TA cloning**).

KAPA HiFi DNA Polymerase is supplied with two uniquely-formulated PCR buffers for optimal performance. Both buffers contain $MgCl_2$ at a 1X concentration of 2 mM. The Fidelity Buffer is recommended for routine high-fidelity PCR, while the GC Buffer is recommended for the amplification of GC-rich and other difficult targets. The additives in the GC Buffer result in a 2-fold decrease in fidelity when compared with the Fidelity Buffer.

Kapa/*Roche* Kit Codes and Components		
KK2500 *07958862001* (20 U)	KAPA HiFi HotStart DNA Polymerase (1 U/µL) KAPA HiFi Fidelity Buffer (5X) KAPA HiFi GC Buffer (5X) $MgCl_2$ (25 mM) KAPA dNTP Mix (10 mM each)	20 µL 1.5 mL 1.5 mL 1.6 mL 40 µL
KK2501 *07958889001* (100 U)	KAPA HiFi HotStart DNA Polymerase (1 U/µL) KAPA HiFi Fidelity Buffer (5X) KAPA HiFi GC Buffer (5X) $MgCl_2$ (25 mM) KAPA dNTP Mix (10 mM each)	100 µL 1.5 mL 1.5 mL 1.6 mL 160 µL
KK2502 *07958897001* (250 U)	KAPA HiFi HotStart DNA Polymerase (1 U/µL) KAPA HiFi Fidelity Buffer (5X) KAPA HiFi GC Buffer (5X) $MgCl_2$ (25 mM) KAPA dNTP Mix (10 mM each)	250 µL 1.5 mL 1.5 mL 1.6 mL 300 µL

Quick Notes

- KAPA HiFi HotStart PCR Kits contain the engineered KAPA HiFi HotStart DNA Polymerase – developed for fast and versatile high-fidelity PCR.
- KAPA HiFi DNA Polymerase has the lowest published error rate of all B-family DNA polymerases (1 error per 3.6 x 10^6 nucleotides incorporated).
- Amplify targets up to 15 kb from genomic DNA or 20 kb from less complex targets.
- KAPA HiFi Buffers contain 2 mM $MgCl_2$ at 1X.
- Use the Fidelity Buffer for routine high-fidelity PCR, and the GC Buffer for GC-rich and other difficult targets.
- Denature at 98°C for 20 sec per cycle.
- Optimal annealing temperatures are typically higher than in other PCR buffer systems. Use an annealing temperature gradient to determine the optimal annealing temperature.
- To ensure the highest fidelity, use high quality DNA and the lowest possible number of cycles.

Product Applications

The KAPA HiFi HotStart PCR Kit is ideally suited for:

- PCR for conventional sequencing (direct sequencing or sequencing of cloned PCR products)
- Amplification of DNA fragments for cloning and protein expression or genomic characterization
- 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** available from **www.kapabiosystems.com**.

Standard PCR Protocol

IMPORTANT! The KAPA HiFi HotStart PCR Kit contains an engineered B-family (proofreading) DNA polymerase and uniquely-formulated buffers, 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

- KAPA HiFi HotStart reactions MUST be set up on ice since the high proofreading activity of the enzyme will result in rapid primer degradation at room temperature.
- 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 KAPA HiFi Buffer (Fidelity or GC)[2]	5.0 µL	1X
10 mM KAPA dNTP Mix	0.75 µL	0.3 mM each
10 µM Forward Primer	0.75 µL	0.3 µM
10 µM Reverse Primer	0.75 µL	0.3 µM
Template DNA[3]	As required	As required
1 U/µL KAPA HiFi HotStart DNA Polymerase	0.5 µL	0.5 U

[1] Reaction volumes may be adjusted between 10–50 µL. For volumes other than 25 µL, scale reagents down proportionally. Reaction volumes >50 µL are not recommended.

[2] KAPA HiFi Buffers contain 2 mM $MgCl_2$ (1X). Additional $MgCl_2$ may be added separately. Use the GC Buffer only if the Fidelity Buffer gives poor results.

[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.

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]	95°C	3 min	1
Denaturation[2]	98°C	20 sec	15–35[6]
Annealing[3,4]	60–75°C	15 sec	
Extension[5]	72°C	15–60 sec/kb	
Final extension	72°C	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] KAPA HiFi Buffers have a higher salt concentration than conventional PCR buffers, which affects DNA melting. To ensure that complex and GC-rich targets are completely denatured, use a temperature of 98°C for denaturation during cycling.

[3] In addition to DNA melting, the high-salt buffers also affect primer annealing. The optimal annealing temperature for a specific primer set is likely to be different (higher) than when used in a conventional PCR buffer. An annealing temperature gradient PCR is recommended to determine the optimal annealing temperature with KAPA HiFi HotStart. If gradient PCR is not feasible, anneal at 65°C as a first approach.

[4] 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 be used.

[5] Use 15 sec extension per cycle for targets ≤1 kb, and 30–60 sec/kb for longer fragments, or to improve yields.

[6] For highest fidelity, use ≤25 cycles. In cases where very low template concentrations or low reaction efficiency results in low yields, 30–35 cycles may be performed to produce sufficient product for downstream applications.

Product Specifications

Shipping, storage and handling

KAPA HiFi HotStart PCR Kits are shipped on dry ice or ice packs, depending on the country of destination. Upon arrival, store kit components at -20°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. KAPA HiFi Buffers contain isostabilizers and may not freeze solidly, even when stored at -20°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 4°C for short-term use (up to 1 month). Return to -20°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 4°C is not recommended. Please note that reagents stored at temperatures above -20°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 KAPA HiFi HotStart DNA Polymerase is confirmed to contain <2% contaminating protein (Agilent Protein 230 Assay). KAPA HiFi 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

Annealing temperature

Due to the high salt concentration of the KAPA HiFi Buffers, the optimal annealing temperature for a given primer set is usually higher when compared to a different buffer system. When using the KAPA HiFi HotStart PCR Kit with a specific primer pair for the first time, determine the optimal annealing temperature with annealing temperature gradient PCR. We recommend a gradient from 60–72°C, although some assays may require even higher annealing temperatures. For assays with optimal annealing temperatures of 68°C or higher, 2-step cycling may be performed at the optimal annealing temperature. Optimal annealing temperatures below 60°C are rare, but may be required when using primers with a high AT content.

If a gradient PCR is not feasible, use an annealing temperature of 65°C as a first approach, and adjust the annealing temperature based on the results obtained:

- If a low yield of only the specific product is obtained, lower the annealing temperature in 1–2°C increments.
- If nonspecific products are formed in addition to the specific product, increase the annealing temperature in 1–2°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**).

NOTE: The optimal annealing temperature for a specific amplicon is typically 5–6°C lower in the GC Buffer than in the Fidelity Buffer.

$MgCl_2$ concentration

KAPA HiFi Buffers contain a final (1X) $MgCl_2$ concentration of 2 mM, which is sufficient for most applications. Applications which are likely to require higher $MgCl_2$ concentrations include long PCR (>10 kb) and AT-rich PCR, as well as amplification using primers with a low GC content (<40%).

GC-rich PCR

For GC-rich amplicons, use the GC Buffer. Alternatively, evaluate the Fidelity Buffer + 5% DMSO. Should neither of these result in successful amplification, perform reactions in both the Fidelity and GC Buffers, adding either 1X KAPA Enhancer 1 (supplied with KAPA2G Robust PCR Kits) or 1 M betaine to determine whether this improves yield and/or specificity.

Primer and Template DNA quality

Another critical factor for successful PCR with KAPA HiFi HotStart is primer design and quality. Primers should be carefully designed to eliminate the possibility of primer-dimer formation and nonspecific annealing as far as possible, and should have a GC content of 40–60%. Primers with GC content >60% may require higher denaturation temperatures and/or longer denaturation times, while primers with 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.

NOTE: 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 to limit degradation and maintain primer quality.

High-quality template DNA is essential for high-fidelity amplification. Degraded, damaged, or sheared template DNA is particularly problematic when amplifying longer fragments (>1 kb). To limit degradation and maintain template quality, always dilute and store DNA in a buffered solution (e.g. 10 mM Tris-HCl, pH 8.0–8.5) instead of PCR-grade water.

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.

TA cloning

DNA fragments generated with the KAPA HiFi HotStart PCR Kit may be used directly for blunt-end cloning, or cloning using restriction endonucleases. For TA cloning of KAPA HiFi HotStart PCR products, first purify the PCR product to remove the KAPA HiFi HotStart DNA Polymerase, as residual proofreading activity will remove any dA-overhangs added during the A-tailing reaction. Perform A-tailing by combining the purified PCR product, 1X *Taq* buffer (with 1.5 mM $MgCl_2$), 0.2 mM dATP and 1 U of *Taq* DNA polymerase and incubating for 5 min at 72°C.

Troubleshooting

Symptoms	Possible causes	Solutions
No amplification or low yield	Cycling protocol	Use the recommended 3–5 min initial denaturation at 95°C, and perform cycle denaturation for 20 sec at 98°C. Increase the extension time to a maximum of 1 min/kb. Increase the number of cycles.
	Annealing temperature is too high	Reduce the annealing temperature by 5°C, or try the GC Buffer. 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 $MgCl_2$. 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. Store and dilute primers in a buffered solution, not water.
	$MgCl_2$	Optimize $MgCl_2$ concentration. AT-rich PCR typically requires more $MgCl_2$.
	dNTPs	dNTP quality is critical. Use only KAPA dNTPs supplied with the kit.
Nonspecific amplification or smearing	Template DNA	Use <100 ng of DNA per reaction, or reduce the number of cycles. 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. 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	Use the GC Buffer, or add 5% DMSO to Fidelity Buffer. Add 1X KAPA Enhancer 1 or 1 M betaine to reactions with Fidelity and/or GC Buffer to facilitate melting of GC-rich templates.
	Enzyme concentration	Do not exceed 0.5 U of KAPA HiFi 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. Store and dilute primers in a buffered solution, not water.

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