KAPA HiFi HotStart ReadyMix PCR Kit

KR0370 – v16.25

This Technical Data Sheet provides product information and a detailed protocol for the KAPA HiFi HotStart ReadyMix PCR Kit.

This document applies to the following kits: 07958927001, 07958935001, 09420398001 and 09420444001.

Contents

Kapa/*Roche* Kit Codes and Components		
KK2601 *07958927001*	KAPA HiFi HotStart ReadyMix (2X)*	1.25 mL
KK2602 *07958935001*	KAPA HiFi HotStart ReadyMix (2X)*	6.25 mL
09420398001	KAPA HiFi HotStart ReadyMix (2X)*	9.6 mL
09420444001 (96-well plate)	KAPA HiFi HotStart ReadyMix (2X)*	96 x 25 µL

* Contains 2.5 mM $MgCl_2$ at 1X

Quick Notes

- KAPA HiFi DNA Polymerase is extensively used in next-generation sequencing (NGS) library amplification. If you are using this product in library construction protocols, you may find KAPA HiFi HotStart Library Amplification Kits more convenient. These kits contain the KAPA HiFi HotStart DNA Polymerase in a ReadyMix formulation, with or without KAPA Library Amplification Primer Mix for the amplification of Illumina® libraries. Please refer to the KAPA HiFi HotStart Library Amplification Kit Technical Data Sheet (KR0408) for details and a standard library amplification protocol.
- KAPA HiFi HotStart ReadyMix PCR Kits contain the engineered KAPA HiFi HotStart DNA Polymerase; developed for fast and versatile high-fidelity PCR.
- Buffer, $MgCl_2$, dNTPs and enzyme are supplied in a convenient 2X ReadyMix – just add primers and template.
- The error rate of KAPA HiFi DNA Polymerase (as determined by 454 sequencing) is 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.
- 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 Description

KAPA HiFi HotStart DNA Polymerase is a 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 with wild-type B-family DNA polymerases. In addition, the ability to amplify long fragments, as well as GC- and AT-rich targets, is significantly improved. The enzyme is combined with a proprietary antibody that inactivates the enzyme until the first denaturation step. This prevents nonspecific amplification during reaction setup, increases sensitivity, and improves reaction efficiency.

In the ReadyMix PCR Kit, KAPA HiFi HotStart DNA Polymerase is supplied in a convenient 2X ReadyMix format, containing all reaction components except primers and template. The ReadyMix contains KAPA HiFi HotStart DNA Polymerase (0.5 U per 25 µL reaction) in a proprietary reaction buffer containing dNTPs (0.3 mM of each dNTP at 1X), $MgCl_2$ (2.5 mM at 1X) and stabilizers.

KAPA HiFi HotStart ReadyMix is designed for routine, high-fidelity PCR of a wide range of targets and fragment sizes. It offers 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 HotStart requires significantly shorter cycling 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 extremely high accuracy during DNA amplification. The error rate of KAPA HiFi HotStart DNA Polymerase (determined by 454 sequencing) is 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 ReadyMix may be used for routine downstream analysis and applications, including restriction enzyme digestion, cloning and sequencing. PCR products generated with KAPA HiFi HotStart ReadyMix are blunt-ended, but may be 3'-dA-tailed for cloning into TA-cloning vectors (see **Important Parameters: TA-cloning**).

Product Applications

The KAPA HiFi HotStart PCR Kit is ideally suited for:

- NGS library amplification
- Amplification of fragments for Sanger sequencing (direct sequencing or sequencing of cloned PCR products)
- Amplification of DNA fragments to be cloned for protein expression or genomic characterization
- Site-directed mutagenesis.

For more information on these and other high-fidelity PCR applications, please refer to KAPA HiFi Application Notes available from sequencing.roche.com.

Product Specifications

Shipping and Storage

The enzymes provided in this kit are temperature sensitive, and appropriate care should be taken during shipping and storage. KAPA HiFi HotStart ReadyMix 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.

Handling

KAPA HiFi HotStart ReadyMix contains 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. Nevertheless, 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.

Plate Handling

Remove the plate from its packaging sleeve and thaw at room temperature or in a suitable cooled reagent block. Place on ice once completely thawed. Always ensure the components have been thoroughly mixed before use. Centrifuge the plate at room temperature (e.g., for 1 minute at 280 x g) to ensure that all liquid is collected in the bottom of wells before the seal is removed. If using only a subset of wells, partially remove the foil seal from the desired area by first using a sterile scalpel to make an incision in the foil. Be careful not to tear the foil unevenly. Do not reuse the partial seal.If you are not using the entire contents of the plate at this time, apply a new adhesive foil seal. Make sure that the foil is properly aligned and fully covers all 96 wells. Use a roller or other appropriate tool to ensure that the foil is evenly applied. Store the re-sealed plate upright at -15°C to -25°C in a constant temperature freezer for subsequent use.

Quality Control

Each batch of KAPA HiFi HotStart DNA Polymerase is confirmed to contain <2% contaminating protein (Agilent Protein 230 Assay). All kit components are subjected to stringent quality control tests, are free of detectable contaminating endonuclease activity, and meets strict requirements with respect to DNA contamination levels. Please contact Technical Support at sequencing.roche.com/support for more information.

Safety Information

Precautions

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material can vary, the operator must optimize pathogen inactivation and follow the appropriate measures according to local safety regulations.
- Do not eat, drink, or smoke in the laboratory area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats, and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online (eLabDoc) or upon request from the local Roche office.

Important Parameters

Annealing temperature

Due to the high salt concentration of the KAPA HiFi HotStart ReadyMix, the optimal annealing temperature for a given primer set is usually different when compared with a different buffer system. When using the ReadyMix with a specific primer pair for the first time, determine the optimal annealing temperature with an 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, two-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 primer pair with KAPA HiFi HotStart ReadyMix is typically 2 – 3°C lower than the optimal annealing temperature in KAPA HiFi Fidelity Buffer, and 2 – 3°C higher than the KAPA HiFi GC Buffer optimal annealing temperature.

$MgCl_2$ concentration

KAPA HiFi HotStart ReadyMix contains a final (1X) $MgCl_2$ concentration of 2.5 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, reactions may be supplemented with 5% DMSO, 1X KAPA Enhancer 1 (supplied with KAPA2G Robust PCR Kits) or 1 M betaine to improve yield and/or specificity.

Primer and Template DNA quality

Another critical factor for successful PCR with KAPA HiFi HotStart ReadyMix 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 ReadyMix may be used directly for blunt-end cloning, or cloning using restriction endonucleases. For TA cloning of KAPA HiFi HotStart ReadyMix 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.

NGS library amplification

NGS library amplification differs from other high-fidelity PCR applications in three noteworthy ways: (i) unlike genomic DNA or plasmids, templates are comprised of highly heterogenous populations of linear DNA or cDNA fragments; (ii) the input copy number is orders of magnitude higher than in "conventional" PCR applications, and (iii) the aim is not to amplify a single amplicon with high specificity, but to amplify a complex collection of library fragments with minimal bias. For important parameters relating to NGS library amplification with KAPA HiFi kits, please refer to the KAPA HiFi HotStart Library Amplification Kit Technical Data Sheet (KR0408).

Standard PCR Protocol

IMPORTANT! The KAPA HiFi HotStart ReadyMix 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 Parameter**s for more information.

IMPORTANT! The reaction setup and cycling conditions below serve as a starting point for standard PCR. If you are using KAPA HiFi HotStart ReadyMix for NGS library amplification, please refer to the instructions in the KAPA HiFi HotStart Library Amplification Kit Technical Data Sheet (KR0408).

Step 1: Prepare the PCR master mix

1.1 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.

1.2 Ensure that all reagents are properly thawed and mixed.

1.3 Prepare a PCR master mix containing the appropriate volume of all reaction components common to all or a subset of reactions to be performed.

1.4 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
2X KAPA HiFi HotStart ReadyMix[2,3]	12.5 µL	1X
10 µM Forward Primer	0.75 µL	0.3 µM
10 µM Reverse Primer	0.75 µL	0.3 µM
Template DNA[4]	As required	As required

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

[2] KAPA HiFi HotStart ReadyMix contains 2.5 mM $MgCl_2$ (1X). Additional $MgCl_2$ may be added separately, but is unlikely to be required.

[3] KAPA HiFi HotStart ReadyMix contains 0.3 mM of each dNTP (1X), and 0.5 U of KAPA HiFi HotStart DNA Polymerase (per 25 µL reaction) in a proprietary reaction buffer.

[4] 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

2.1 Transfer the appropriate volumes of PCR master mix, template and primer to individual PCR tubes or wells of a PCR plate.

2.2 Cap or seal individual reactions, mix and centrifuge briefly.

Step 3: Run the PCR

3.1 Perform PCR with the following cycling protocol:

Step	Temperature	Duration	Cycles
Initial denaturation[1]	95ºC	3 min	1
Denaturation[2]	98ºC	20 sec	15 – 35[6]
Annealing[3,4]	60 – 75ºC	15 sec	
Extension[5]	72ºC	15 – 60 sec/kb	
Final extension	72ºC	1 min/kb	1

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

[2] KAPA HiFi HotStart ReadyMix has a higher salt concentration than conventional PCR ready-mixes, 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, high salt also affects primer annealing. The optimal annealing temperature for a specific primer set is likely to be different (higher) than when used in a conventional PCR ready-mix. An annealing temperature gradient PCR is recommended to determine the optimal annealing temperature with the KAPA HiFi HotStart ReadyMix. 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.

Appendix A - Troubleshooting

Symptoms	Key parameters	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. 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.
	$MgCl_2$ concentration	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. 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	Add 5% DMSO, 1X KAPA Enhancer 1 or 1 M betaine to reactions to facilitate melting of GC-rich templates.
	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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