KAPABIOSYSTEMS

Technical Data Sheet

KAPA HiFi HotStart Library Amplification Kit

Illumina® Platforms

KR0408 – v9.19

This Technical Data Sheet provides product information and a detailed protocol for the KAPA HiFi HotStart Library Amplification Kits.

This documents applies to KAPA HiFi HotStart Library Amplification Kits (07958978001 and 07958986001), KAPA HiFi HotStart Library Amplification Kits (ReadyMix only) (07958943001, 07958951001 and 07958960001), and the KAPA Library Amplification Primer Mix Kit (07958994001).

Contents

Kapa/*Roche* Kit Codes and Components		
KK2620 *07958978001* (50 reactions)	KAPA HiFi HotStart ReadyMix (2X) KAPA Library Amplification Primer Mix (10X)	1.25 mL 0.25 mL
KK2621 *07958986001* (250 reactions)	KAPA HiFi HotStart ReadyMix (2X) KAPA Library Amplification Primer Mix (10X)	6.25 mL 1.25 mL
KK2610 *07958943001* (10 reactions)	KAPA HiFi HotStart ReadyMix (2X)	0.25 mL
KK2611 *07958951001* (50 reactions)	KAPA HiFi HotStart ReadyMix (2X)	1.25 mL
KK2612 *07958960001* (250 reactions)	KAPA HiFi HotStart ReadyMix (2X)	6.25 mL
KK2623 *07958994001* (250 reactions)	KAPA Library Amplification Primer Mix (10X)	1.25 mL

Quick Notes

- KAPA HiFi DNA Polymerase is ideally suited for the amplification of next-generation sequencing (NGS) libraries, but is also designed for robust, high-fidelity PCR applications. If you are using this product for an application in which reaction optimization is important, you may want to consider using a KAPA HiFi kit in which all components are supplied as separate solutions. Please refer to the KAPA HiFi PCR Kit Technical Data Sheet (KR0368; non-HotStart enzyme) or the KAPA HiFi HotStart PCR Kit Technical Data Sheet (KR0369; HotStart enzyme) for details and a standard PCR protocol.
- KAPA HiFi HotStart ReadyMix (2X) contains the engineered KAPA HiFi HotStart DNA Polymerase that is specifically designed to minimize amplification bias, while maintaining extremely high fidelity.
- The error rate of KAPA HiFi DNA Polymerase (as determined by 454 sequencing) is 1 error per 3.6 x 10^6 nucleotides incorporated.
- The KAPA Library Amplification Primer Mix (10X) contains primers complementary to the P5 and P7 regions of Illumina adapters and indexing primers. The primer mix is formulated to limit primer depletion and over-amplification.
- Kits without primers (07958943001, 07958951001 and 07958960001) are available for library amplification with user-supplied primers.

KAPA HiFi HotStart Library Amplification Kit

Illumina® Platforms Technical Data Sheet

Product Description

KAPA HiFi HotStart Library Amplification Kits for Illumina platforms are designed for the amplification of next-generation sequencing (NGS) libraries prepared for Illumina sequencing.

In order to maximize sequence coverage uniformity, it is critical to minimize library amplification bias. Amplification bias occurs when a DNA polymerase is unable to amplify all targets within a complex population of library DNA with equal efficiency. KAPA HiFi DNA Polymerase is a B-family DNA polymerase engineered for increased processivity, extremely high fidelity and low-bias, and is the reagent of choice for NGS library amplification.[1,2,3,4] KAPA HiFi HotStart has 5'→3' polymerase and 3'→5' exonuclease (proofreading) activities. The error rate of KAPA HiFi HotStart DNA Polymerase is 2.8×10^{-7} errors/base, equivalent to 1 error per 3.5×10^{6} nucleotides incorporated. 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.

KAPA HiFi HotStart Library Amplification Kits include KAPA HiFi HotStart ReadyMix (2X), a ready-to-use PCR mix comprising all the components for library amplification; except primers and template. Kits also include the KAPA Library Amplification Primer Mix (10X), designed for the high-efficiency amplification of Illumina libraries flanked by adapters containing the P5 and P7 flow cell sequences.

1. Oyola, S.O., et al., *BMC Genomics* **13**, 1 (2012).
2. Quail, M.A., et al., *Nature Methods* **9**, 10 (2012).
3. Quail, M.A., et al., *BMC Genomics* **13**, 341 (2012).
4. Ross, M.G., et al., *Genome Biology* **14**, R51 (2013).

Product Applications

KAPA HiFi HotStart Library Amplification Kits for Illumina platforms are ideally suited for high-efficiency, high-fidelity, low-bias amplification of libraries prior to Illumina sequencing. This includes libraries prepared for:

- whole-genome shotgun sequencing
- exome or targeted sequencing (pre- and post-capture amplification)
- RNA-seq
- ChIP-seq
- other sequencing applications

KAPA HiFi DNA Polymerase may also be used for other high-fidelity PCR applications. Please visit www.sequencing.roche.com to select the most appropriate KAPA HiFi formulation for your application.

KAPA HiFi DNA Polymerase is not suitable for the amplification of templates containing uracil, such as bisulfite-treated DNA. For such templates, KAPA HiFi HotStart Uracil+ ReadyMix PCR Kits should be used.

Product Specifications

Shipping and Storage

The components provided in this kit are temperature sensitive, and appropriate care should be taken during shipping and storage. KAPA HiFi HotStart Library Amplification Kits are shipped on dry ice or ice packs, depending on the destination country. Upon receipt, store the entire kit at -15°C to -25°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 label.

Handling

Keep all reaction components and master mixes on ice whenever possible during handling. KAPA HiFi HotStart ReadyMix (2X) may not freeze completely, even when stored at -15°C to -25°C. Nevertheless, always ensure that the KAPA HiFi HotStart ReadyMix and KAPA Library Amplification Primer Mix (10X) is fully thawed and thoroughly 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.

Quality Control

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

Important Parameters

Cycle Number

Excessive library amplification should be avoided to minimize the following adverse effects:

- increased duplicate reads
- uneven coverage depth and sequence dropout
- chimeric library inserts
- nucleotide substitutions
- heteroduplex formation.

To minimize over-amplification and associated unwanted artifacts, the number of amplification cycles should be optimized to produce a sufficient amount of amplified library for the next step in the workflow (e.g. hybridization capture or sequencing), plus the amount needed for library QC and/or archiving. Depending on the sequencing application and degree of multiplexing, 100 ng – 1 µg of amplified library may be required.

Table 1 indicates the number of cycles typically required to generate 100 ng or 1 µg of amplified library DNA for libraries prepared with the KAPA Hyper Prep and KAPA HyperPlus Kits; the actual optimal number of cycles may be higher, depending on the reagents and protocol used for library construction, and the quality of the input DNA. For libraries prepared from FFPE DNA or other challenging samples, or libraries with a broad fragment size distribution, 1 – 3 additional cycles may be required. Size selection of libraries at any part in the library construction process results in significant loss of material and as a result, 2 – 4 additional cycles are required for workflows which include a size-selection step prior to library amplification.

Please consult the relevant library preparation kit Technical Data Sheet for specific recommendations.

Table 1: Recommended library amplification cycles to generate 100 ng or 1 µg of amplified library, constructed from different amounts of input DNA with a KAPA HyperPrep or KAPA HyperPlus Kit

Input DNA (into library construction)	Number of cycles required to generate 100 ng – 1 µg			
	KAPA Hyper Prep		KAPA HyperPlus	
	100 ng library	1 µg library	100 ng library	1 µg library
1 µg	0	1 – 2	0	0 – 1
500 ng	0	2 – 4	0	2 – 3
250 ng	1 – 2	4 – 6	0 – 1	3 – 5
100 ng	2 – 3	6 – 7	0 – 2	5 – 6
50 ng	3 – 5	7 – 8	3 – 5	7 – 8
25 ng	5 – 7	8 – 10	5 – 7	8 – 10
10 ng	7 – 9	11 – 13	7 – 9	11 – 13
5 ng	9 – 11	13 – 14	9 – 11	13 – 14
2.5 ng	11 – 13	14 – 16	11 – 13	14 – 16
1 ng	13 – 15	17 – 19	13 – 15	17 – 19

Primer Quality and Concentration

To achieve the highest amplification efficiency and avoid primer depletion, it is critical to use an optimal concentration of high-quality primers. Primers should be used at a final concentration of 0.5 – 2 µM each. For libraries constructed from ≥100 ng input DNA, the highest final concentration (2 µM of each primer) is recommended.

Library amplification primers should modified to include a phosphorothioate bond at the 3'-terminal of each primer (to prevent degradation by the strong proofreading activity of KAPA HiFi DNA Polymerase). Always store and dilute primers in a buffered solution (e.g., 10 mM Tris-HCl, pH 8.0 – 8.5), and limit the number of freeze-thaw cycles. To achieve the latter, store primers at 4°C for short-term use, or as single-use aliquots at -20°C.

Primer Depletion and Library Over-amplification

In library amplification reactions (set up according to the recommended protocol), primers are typically depleted before dNTPs. When DNA synthesis can no longer take place due to primer depletion, subsequent rounds of DNA denaturation and annealing result in the separation of complementary DNA strands, followed by imperfect annealing to non-complementary partners. This presumably results in the formation of so-called "daisy-chains" or tangled knots, comprising large assemblies of improperly annealed, partially double-stranded, heteroduplex DNA. These species migrate slower and are observed as secondary, higher molecular weight peaks during the electrophoretic analysis of amplified libraries. However, they are typically comprised of library molecules of the desired length, which are separated during denaturation prior to target enrichment (using hybridization capture protocols) or cluster amplification. Since these heteroduplexes contain significant portions of single-stranded DNA, over-amplification leads to the under-quantification of library molecules with assays employing dsDNA-binding dyes. qPCR-based library quantification methods, such as the KAPA Library Quantification assay, employ successive rounds of denaturation and amplification to quantify DNA, thereby providing a more accurate measurement of the amount of adapter-ligated molecules; even if the library was over-amplified.

Please refer to the **KAPA NGS Library Preparation Technical Guide** for a more detailed discussion of factors that can affect the efficiency of library amplification, and the impact of over-amplification on library quantification.

Optimization of Library Amplification

The quantification of adapter-ligated libraries (prior to library amplification) can greatly facilitate the optimization of library amplification parameters, particularly when a library construction workflow is first established. With the KAPA Library Quantification Kit, the amount of template DNA (adapter-ligated molecules) available for library amplification can be determined accurately. From there, the number of amplification cycles needed to achieve a specific yield of amplified library can be predicted theoretically. Please contact Technical Support at sequencing.roche.com/support for a calculator designed to assist with these calculations.

Post-amplification Cleanup

- This protocol has been validated for use with either KAPA Pure Beads or Agencourt® AMPure® XP reagent (Beckman Coulter®). Solutions and conditions for DNA binding may differ if other beads are used.
- Observe all the storage and handling recommendations for KAPA Pure Beads or AMPure XP reagent. Equilibration to room temperature is essential to achieve specified size distribution and yield of libraries.
- Beads will settle gradually; always ensure that they are fully resuspended before use.
- ***To ensure optimal DNA recovery, it is critical that the DNA and the cleanup beads are thoroughly mixed*** (by vortexing or extensive up-and-down pipetting) before the DNA binding incubation.
- Bead incubation times are guidelines only, and may be modified/optimized according to current protocols, previous experience, specific equipment and samples in order to maximize library construction efficiency and throughput.
- The time required for complete capture of beads varies according to the reaction vessel and magnet used. It is important not to discard or transfer any beads with the removal or transfer of supernatant. Capture times should be optimized accordingly.
- The volumes of 80% ethanol used for bead washes may be adjusted to accommodate smaller reaction vessels and/or limited pipetting capacity, but it is important that the beads are entirely submerged during the wash steps. ***Always use freshly prepared 80% ethanol.***
- It is important to remove all ethanol before proceeding with subsequent reactions. However, over-drying of beads may make them difficult to resuspend, resulting in a dramatic loss of DNA. With optimized aspiration of ethanol, drying of beads for 3 – 5 min at room temperature should be sufficient. ***Drying of beads at 37°C is not recommended.***
- Where appropriate, DNA should be eluted from beads in elution buffer (10 mM Tris-HCl, pH 8.0 – 8.5). Elution of DNA in PCR-grade water is not recommended, as DNA is unstable in unbuffered solutions. However, libraries constructed for hybridization target capture that require drying of DNA prior to probe hybridization must be eluted and stored in PCR-grade water. Purified DNA in elution buffer should be stable at 4°C for 1 – 2 weeks, or at -20°C for long-term storage. The long-term stability of library DNA at -20°C depends on a number of factors, including library concentration. Always use low DNA-binding tubes for long-term storage, and avoid excessive freezing and thawing.
- Please refer to the **KAPA Pure Beads Technical Data Sheet (KR1245)** for additional detailed information and protocols.

Library Amplification Protocol

Step 1: Library Amplification

The KAPA Library Amplification Primer Mix (10X) is designed to eliminate or delay primer depletion during library amplification reactions performed with KAPA HiFi HotStart ReadyMix (2X). The primer mix is suitable for the amplification of all Illumina libraries flanked by the P5 and P7 flow cell sequences. Primers are supplied at a 10X concentration of 20 µM each, and have been formulated as previously described. User-supplied primer mixes may be used in combination with incomplete or custom adapters. For guidelines on the formulation of user-supplied library amplification primers, please contact Technical Support at sequencing.roche.com/support.

1.1 Assemble each library amplification reaction as follows:

Component	Volume
KAPA HiFi HotStart ReadyMix (2X)	25 µL
KAPA Library Amplification Primer Mix (10X)*	5 µL
Adapter-ligated library DNA	20 µL
Total volume:	**50 µL**

*Or another suitable 10X library amplification primer mix. The recommended final concentration of each primer in the library amplification reaction is 0.5 – 2 µM.

1.2 Mix thoroughly and centrifuge briefly.

1.3 Amplify using the following cycling protocol:

Step	Temp.	Duration	Cycles
Initial denaturation	98°C	45 sec	1
Denaturation	98°C	15 sec	Minimum required for optimal amplification[2]
Annealing[1]	60°C	30 sec	
Extension	72°C	30 sec	
Final extension	72°C	1 min	1
HOLD	4°C	∞	1

[1] Optimization of the annealing temperature may be required for non-standard (i.e., other than Illumina TruSeq®) adapter/primer combinations.

[2] The optimal cycling number will depend upon the volume and concentration of adapter-ligated, size separated, purified library DNA added to each enrichment PCR reaction.

1.4 Proceed directly to **Post-amplification Cleanup** (step 2).

Step 2: Post-amplification Cleanup

2.1 In the library amplification plate/tube(s), perform a 1X bead-based cleanup by combining the following:

Component	Volume
Library amplification reaction product	50 µL
KAPA Pure Beads*	50 µL
Total volume:	**100 µL**

*Or Agencourt® AMPure® XP reagent (Beckman Coulter®)

2.2 Mix thoroughly by vortexing and/or pipetting up and down multiple times.

2.3 Incubate the plate/tube(s) at room temperature for 5 – 15 min to bind DNA to the beads.

2.4 Place the plate/tube(s) on a magnet to capture the beads. Incubate until the liquid is clear.

2.5 Carefully remove and discard the supernatant.

2.6 Keeping the plate/tube(s) on the magnet, add 200 µL of freshly-prepared 80% ethanol.

2.7 Incubate the plate/tube(s) on the magnet at room temperature for ≥30 sec.

2.8 Carefully remove and discard the ethanol.

2.9 Keeping the plate/tube(s) on the magnet, add 200 µL of 80% ethanol.

2.10 Incubate the plate/tube(s) on the magnet at room temperature for ≥30 sec.

2.11 Carefully remove and discard the ethanol. Try to remove all residual ethanol without disturbing the beads.

2.12 Dry the beads at room temperature for 3 – 5 min, or until all of the ethanol has evaporated. ***Caution: over-drying the beads may result in reduced yield.***

2.13 Remove the plate/tube(s) from the magnet.

2.14 Thoroughly resuspend the beads in an appropriate volume of elution buffer (10 mM Tris-HCl, pH 8.0 – 8.5) or PCR-grade water if proceeding to hybridization target capture that requires drying of DNA.

2.15 Incubate the plate/tube(s) at room temperature for 2 min to elute DNA off the beads.

2.16 Place the plate/tube(s) on a magnet to capture the beads. Incubate until the liquid is clear.

2.17 Transfer the clear supernatant to a new plate/tube(s). Store purified, amplified libraries at 2°C to 8°C for 1 – 2 weeks, or at -15°C to -25°C.

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