KAPA Library Amplification Kit

Kit codes and components	
KK2610 Sample kit	KAPA HiFi HotStart ReadyMix (2X) 1 x 0.25 ml (10 x 50 µl rxns)
KK2611 50 reactions	KAPA HiFi HotStart ReadyMix (2X) 1 x 1.25 ml (50 x 50 µl rxns)
KK2612 250 reactions	KAPA HiFi HotStart ReadyMix (2X) 1 x 6.25 ml (250 x 50 µl rxns)

Storage, handling and specifications

Store all components at -20 °C for long-term use. Please refer to Section 4 for full details.

Quick Notes

- KAPA Library Amplification Kits contain the novel KAPA HiFi DNA Polymerase, engineered for increased processivity and high fidelity.
- KAPA HiFi HotStart ReadyMix is specifically designed to minimize the effects of amplification bias whilst maintaining industry leading fidelity.
- The optimal cycling number is determined by the volume and concentration of adaptor-ligated library DNA added to each enrichment PCR reaction. Typically this is in the 10-18 cycle range but may require optimization.
- When using custom primers that differ in sequence from those listed in Table 1, we recommend performing gradient PCR to optimize the annealing temperature.
- The kit is compatible with the Nextera™ Sample Preparation protocol. Use 98 °C denaturation temperature.

1. Product Description

High fidelity PCR is used to selectively enrich library fragments carrying appropriate adaptor sequences and to amplify the amount of DNA prior to sequencing. During PCR enrichment of libraries, it is critical that amplification bias is kept to a minimum to ensure uniform sequence coverage. This enables efficient sequencing where the highest overall coverage can be achieved from the least amount of total sequence. KAPA Library Amplification Kits are designed for low bias, high fidelity PCR representing the method of choice for the amplification of next-generation sequencing libraries.

KAPA Library Amplification Kits contain KAPA HiFi HotStart ReadyMix (2X), a ready-to-use cocktail containing all components for PCR, except primers and template. The 2X ReadyMix contains KAPA HiFi HotStart DNA Polymerase in a proprietary reaction buffer including dNTPs and $MgCl_2$ (2.5 mM at 1X).

KAPA HiFi HotStart DNA Polymerase is an antibody-based hot start formulation of KAPA HiFi DNA Polymerase, a novel B-family DNA polymerase exhibiting industry-leading performance in comparison with other high-fidelity (B-family) DNA polymerases and polymerase blends. KAPA HiFi DNA Polymerase was engineered for increased affinity to DNA, without the need for accessory protein domains. The intrinsic high processivity of the enzyme results in significant improvements in yield, sensitivity, speed, target length and the ability to amplify difficult amplicons. These enhancements result in lower amplification bias which leads to more uniform sequence coverage. In the HotStart formulation, a proprietary antibody inactivates the polymerase 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 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 incorporates 1 error per 3.6 x 10^6 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 ReadyMix may be used for routine downstream analyses or applications, including restriction enzyme digestion 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.

2. Applications

KAPA HiFi HotStart ReadyMix (2X) is ideally suited to the amplification of complex genomic libraries and enrichment of targeted capture sequences where:

- Low amplification bias is required in order to maintain genome representation
- High fidelity is required to identify single nucleotide polymorphisms (SNPs)

For more information please refer to the KAPA Library Amplification Application Note available at **www.kapabiosystems.com**.

3. Library amplification protocol

1: Preparation

- Thaw the primers required for PCR enrichment (see Table 1 for details) and a tube of KAPA HiFi HotStart Ready Mix (2X) at room temperature.
- Briefly centrifuge the thawed KAPA HiFi HotStart Ready Mix (2X) and primer tubes for 5 seconds at 600 xg.
- Thaw and briefly centrifuge the adaptor-ligated, size-separated purified library DNA for 5 seconds at 600 xg.
- Pre-program the thermal cycler using the recommended cycling protocol supplied in Table 1 for the specific type of Illumina library.

2: Reaction setup

In order to maintain optimal library diversity it is necessary to add sufficient adaptor-ligated library DNA to each enrichment PCR reaction. The optimal cycle number is dependant on the volume and concentration of library material added to each 50 uL PCR reaction. Titration PCR may be performed to optimize the yield prior to performing the preparative enrichment PCR reaction/s.

To each reaction add the following components changing tips after each pipetting step. Consult Table 1 for the suggested reaction setup for specific library preparation protocols.

- 25 µL KAPA HiFi HotStart Ready Mix (2X)
- Primer mix or each individual primer
- Purified adaptor-ligated library DNA
- Make up to 50 µL with PCR-grade water
- Seal each reaction, mix gently and centrifuge for 5 seconds at 600 xg

3: Cycling protocol

- Refer to Table 1 for the thermal cycling protocol for specific library types.

4: Clean up PCR

- After enrichment PCR, clean up each reaction using either Agencourt AMPure XP beads (Beckman Coulter Genomics part # A63881) or Qiagen MinElute PCR purification kit (Qiagen, part # 28004).

5: Validate library

- To verify the size of the PCR enriched fragments, check the size distribution by performing gel electrophoresis.
- Use the appropriate KAPA Library Quantification Kit (KK4824, KK4835, KK4844, KK4854) to accurately quantify the number of PCR-competent molecules. Accurate quantification of amplifiable library molecules is critical for the efficient use of the Illumina sequencing platforms. Overestimation of library concentration results in lower cluster density after bridge PCR. Underestimation of library concentration results in too many clusters on the flow cell, which can lead to poor cluster resolution. Both scenarios result in suboptimal sequencing capacity. Accurate library quantification is equally important when pooling indexed libraries for multiplexed sequencing to ensure equal representation of each library.

For advanced troubleshooting or assistance with reaction setup or optimization e-mail **support@kapabiosystems.com**.

3. Library amplification protocol (cont.)

Table 1. Recommended reaction setup and cycling parameters for KAPA HiFi HotStart ReadyMix (2X) reactions:

Type of Illumina Library	Reaction Setup			Cycling Protocol	
	Component	Final Conc.	Volume/50 µL rxn	Step	Duration and Temperature
Genomic DNA ChIP	PCR grade water		As needed	Denaturation	45 sec at 98 °C
	2X KAPA HiFi HS RM	1X	25 µL	Cycling*	15 sec at 98 °C 30 sec at 65 °C 30 sec at 72 °C
	PCR Primer 1.1 PCR Primer 2.1	500 nM 500 nM	1 µL 1 µL		
	Library DNA		As needed	Final Extension	1 min at 72 °C
PE	PCR grade water		As needed	Denaturation	45 sec at 98 °C
	2X KAPA HiFi HS RM	1X	25 µL	Cycling*	15 sec at 98 °C 30 sec at 65 °C 30 sec at 72 °C
	PE PCR Primer 1.0 PE PCR Primer 2.0	500 nM 500 nM	1 µL 1 µL		
	Library DNA		As needed	Final Extension	1 min at 72 °C
PE Multiplex	PCR grade water		As needed	Denaturation	45 sec at 98 °C
	2X KAPA HiFi HS RM	1X	25 µL	Cycling*	15 sec at 98 °C 30 sec at 65 °C 30 sec at 72 °C
	PE PCR Primer InPE 1.0 PE PCR Primer InPE 2.0 PCR Primer Index 1 - 12	500 nM 10 nM 500 nM	1 µL 1 µL 1 µL		
	Library DNA		As needed	Final Extension	1 min at 72 °C
GEX Small RNA	PCR grade water		As needed	Denaturation	45 sec at 98 °C
	2X KAPA HiFi HS RM	1X	25 µL	Cycling*	15 sec at 98 °C 30 sec at 60 °C 15 sec at 72 °C
	Primer GX1 Primer GX2	500 nM 500 nM	0.5 µL 0.5 µL		
	Library DNA		As needed	Final Extension	1 min at 72 °C
TruSeq DNA TruSeq RNA	2X KAPA HiFi HS RM	1X	25 µL	Denaturation	45 sec at 98 °C
	PCR Primer Cocktail (PPC)	500 nM each	5 µL	Cycling*	15 sec at 98 °C 30 sec at 60 °C 30 sec at 72 °C
	Library DNA		20 µL		
				Final Extension	1 min at 72 °C
Nextera™	2X KAPA HiFi HS RM	1X	25 µL	Initial Extension Denaturation	3 min at 72 °C 30 sec at 98 °C
	50X Nextera Primer Cocktail Index1 primer Index1 primer		5 µL 5 µL 5 µL	5 - 9 Cycles*	10 sec at 98 °C 30 sec at 63 °C 3 min at 72 °C
	Tagmented Library		10 µL	Hold	10 °C

* The optimal cycling number is determined by the volume and concentration of adaptor-ligated, size-separated purified library DNA added to each enrichment PCR reaction. Typically this is in the 5 -18 cycle range but may require optimization depending on workflow.

4. Storage, handling and specifications

4.1 Shipping, storage and handling

KAPA Library Amplification 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.

The KAPA HiFi HotStart ReadyMix contains isostabilizers and may not freeze solidly, even when stored at -20 °C. Nevertheless, always ensure that the KAPA HiFi HotStart ReadyMix is fully thawed and has been vortexed before use.

KAPA HiFi HotStart ReadyMix (2X) may be stored at 4 °C for regular, short-term use (up to 1 month). Provided that it has been handled carefully and not contaminated, the ReadyMix 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.

4.2 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. Each batch of ReadyMix is subjected to stringent quality control tests, is free of contaminating exo- and endonuclease activities and meets strict requirements with respect to DNA contamination.

4.3 Product use limitations and licenses

KAPA Library Amplification 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.

For technical support please contact **support@kapabiosystems.com**

KAPABIOSYSTEMS
www.kapabiosystems.com

Boston, Massachusetts, United States
600 West Cummings Park, Suite 2250
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