KAPA HTP Library Preparation Kit Illumina® platforms

KR0426 – v3.13

This Technical Data Sheet provides product information and a detailed protocol for the KAPA HTP Library Preparation Kit (Illumina® platforms), product codes KK8234 and KK8235.

Contents

Kit Codes and Components

KK8234 **KK8235** 96 libraries	KAPA End Repair Buffer (10X)	1.2 ml
	KAPA End Repair Enzyme Mix	600 µl
	KAPA A-Tailing Buffer (10X)	650 µl
	KAPA A-Tailing Enzyme	360 µl
	KAPA Ligation Buffer (5X)	1.3 ml
	KAPA DNA Ligase	600 µl
	KAPA PEG/NaCl SPRI® Solution	40 ml
	KAPA HiFi HotStart ReadyMix (2X)*	3 ml

*KK8235 is available for PCR-free workflows and does not contain KAPA HiFi HotStart ReadyMix for library amplification.

Quick Notes

- The protocol provided in this document is a generic prototype, and may require additional tailoring and optimization.
- The process workflow (p. 9) provides an overview of the library construction process and options for size selection.
- The **KAPA NGS Library Preparation Technical Guide** contains more detailed information about library construction parameters, and may facilitate protocol development and optimization.
- Separate, concentrated enzyme formulations and reaction buffers for end repair, A-tailing, and ligation provide the best combination of product stability, convenience, and efficiency.
- Adapters and PCR primers are not supplied with this kit, and can be obtained from any reputable oligonucleotide vendor.
- SPRI® beads are not included in the kit, but the PEG/NaCl SPRI® Solution required for "with-bead" reaction cleanups is provided.
- Generous reagent excesses are supplied to accommodate the dead volumes required for automated liquid handling.
- A single kit per batch of 96 samples simplifies reagent handling and inventory control.

Product Description

The KAPA HTP Library Preparation Kit is designed for high-throughput library construction for Illumina® sequencing, starting from fragmented, double-stranded DNA. The kit provides all of the enzymes and reaction buffers required for the following steps of library construction:

1. **End repair,** which produces blunt-ended, 5'-phosphorylated fragments.
2. **A-tailing,** during which dAMP is added to the 3'-ends of blunt-ended dsDNA library fragments.
3. **Adapter ligation,** during which dsDNA adapters with 3'-dTMP overhangs are ligated to 3'-A-tailed library fragments.
4. **Library amplification (optional),** which employs PCR to amplify library fragments carrying appropriate adapter sequences on both ends.

The kit has been validated for library construction from 100 ng – 5 µg of human genomic DNA for whole-genome shotgun sequencing or targeted sequencing by solution hybrid selection (capture). For smaller genomes, or lower complexity samples, such as ChIP DNA, amplicons, or cDNA (for RNA-seq), successful library construction has been achieved from low nanogram to picogram quantities (≥100 pg) of input DNA.

The kit provides all of the enzymes and buffers required for library construction and amplification, but does not include adapters, PCR primers or SPRI® beads. Enzyme formulations and reaction buffers for end repair, A-tailing and ligation are supplied in convenient, concentrated formats.

Efficient, cost-effective and automation-friendly reaction cleanups and higher recovery of input DNA are achieved through implementation of the "with-bead" strategy developed at The Broad Institute of MIT & Harvard and Foundation Medicine[1]. The kit includes PEG/NaCl SPRI® (Solid Phase Reversible Immobilization) Solution for this purpose.

In order to maximize sequence coverage uniformity, it is critical to minimize library amplification bias. KAPA HiFi DNA Polymerase has been designed for low-bias, high-fidelity PCR, and is the reagent of choice for NGS library amplification[2, 3, 4]. The KAPA HTP Library Preparation Kit (KK8234) includes KAPA HiFi HotStart ReadyMix (2X), a ready-to-use PCR mix comprising all the components for library amplification, except primers and template. A kit without an amplification module (KK8235) is available for PCR-free workflows. These kits can also be combined with KAPA HiFi Real-Time Library Amplification Kits (KK2701 and KK2702), or with KAPA HiFi HotStart Uracil+ ReadyMix (KK2801 and KK2802) for the amplification of libraries that have undergone bisulfite-treatment.

1. Fisher, S. *et al. Genome Biology* **12**, R1 (2011).
2. Oyola, S.O. *et al. BMC Genomics* **13**, 1 (2012).

Product Applications

The KAPA HTP Library Preparation Kit is ideally suited for high-throughput NGS library construction workflows that involve end repair, A-tailing, adapter ligation, and library amplification (optional). The kit has been tailored to accommodate the specific requirements of automated liquid handling, and the protocol may be adapted for incorporation into workflows for a wide range of NGS applications, including:

- Whole-genome shotgun sequencing
- Targeted sequencing by solution hybrid selection (i.e. exome or custom capture using the Roche Nimblegen™, Agilent SureSelect, Illumina® TruSeq™, or IDT xGen™ Lockdown™ Probes systems)
- ChIP-seq
- RNA-seq
- Methyl-seq (in combination with the KAPA HiFi HotStart Uracil+ ReadyMix)Library Amplification ReadyMix)

Specific guidelines for the construction of libraries for target enrichment using the Roche Nimblegen™ SeqCap EZ system may be found in Appendix 1.

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 Library Preparation Kits are shipped on dry ice or ice packs, depending on the country of destination. Upon receipt, immediately store enzymes and reaction buffers (including PEG/NaCl SPRI® Solution) at -20 °C in a constant-temperature freezer. When stored under these conditions and handled correctly, the kit components will retain full activity until the expiry date indicated on the kit label.

Handling

Always ensure that components have been fully thawed and thoroughly mixed before use. Keep all enzyme components and master mixes on ice as far as possible during handling and preparation. KAPA HiFi HotStart ReadyMix (2X) contains isostabilizers and may not freeze completely, even when stored at -20 °C. Nevertheless, always ensure that the KAPA HiFi HotStart ReadyMix is fully thawed and thoroughly mixed before use. PEG/NaCl SPRI® Solution does not freeze at -20 °C, but should be equilibrated to room temperature and thoroughly mixed before use.

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. Please contact

Important Parameters

High-throughput library construction workflows must be tailored and optimized to accommodate specific experimental designs, sample characteristics, sequencing applications, and equipment. The protocol provided in this document is a generic prototype, and there are many parameters which may be adjusted to optimize performance, efficiency, and cost-effectiveness.

In addition to the information in this section, please consult the **KAPA NGS Library Preparation Technical Guide** and/or contact **support@kapabiosystems.com** for further guidelines when designing or optimizing your library construction workflow.

Automated library construction

The library construction protocol described in this document can be carried out manually, and most protocol development and validation work is usually done this way. Although it is possible to achieve moderately high sample throughput by using multi-channel pipettes and 96-well plates, automated liquid handling is indispensable for high-throughput NGS production lines, and automating a validated manual library construction protocol can represent a significant challenge.

In addition to increased sample throughput, automation may be expected to provide additional advantages such as improved reproducibility and process control. Nevertheless, automation may result in slightly lower yields and/or different size distributions when compared with manual library construction performed by a skilled, experienced and attentive technician. Most often, these discrepancies can be minimized through careful selection of appropriate hardware and plasticware, and by optimizing liquid handling parameters such as aspiration speeds and volumes in automation scripts.

Kapa Biosystems does not supply automated liquid handling equipment, but we are constantly working in partnership with automation solution providers and customers to develop and validate optimized, automated methods for liquid handling platforms suitable for use in NGS library construction. Please contact **support@kapabiosystems.com** for more information about using this kit with your particular automated liquid handling system.

Reaction setup

This kit is intended for high-throughput library construction, and the protocol is therefore designed to be automation-friendly. For this reason, and to enable a streamlined “with-bead” strategy, reaction components are combined into master mixes, rather than dispensed separately into individual reactions. When processing multiple samples, prepare 10 – 20% excess of each master mix. When processing batches of 48 or more

reagents (adapters, AMPure® XP reagent, SPRI® Solution, 80% ethanol and elution buffer), the required excess may vary from one specific liquid handling system to another. Please refer to Section 1 of the Protocol for more details.

96-well PCR plates are typically used for high-throughput library construction. The maximum working volume in these plates is usually ~200 µl, and this is accounted for in this protocol. It may be possible to employ 96-well plates with larger working volumes or deep well plates to accommodate larger reaction volumes for special applications. Always use plastics that are certified to be free of DNAses, RNAses, and nucleases. Low DNA-binding plastics are recommended. When selecting the most appropriate plasticware for your workflow, consider compatibility with:

- the plate gripper and other components of your liquid handling system.
- the magnet used during SPRI® bead manipulations.
- vortex mixers and centrifuges, where appropriate.
- heating blocks or thermocyclers used for reaction incubations and/or library amplification.

Safe stopping points

The library construction process, from end repair to final, amplified library, can be performed in 4 – 8 hours, depending on the specific workflow and number of samples being processed. Automated methods are typically designed to complete the process from end repair to post-ligation processing in an uninterrupted manner, with minimal user intervention. However, the protocol may be paused safely after any of the bead cleanup steps, as described below:

- After the end repair cleanup (Steps 3.1 – 3.13), resuspend the washed beads in 20 µl of 1X A-Tailing Buffer (without enzyme; Step 4.1), and store the reactions at 4 °C for up to 24 hours.
- After the A-tailing cleanup (Steps 5.1 – 5.13), resuspend the washed beads in 20 µl of 1X Ligation Buffer (without enzyme or adapter; Step 6.1), and store the reactions at 4 °C for up to 24 hours.
- After the first post-ligation cleanup (Steps 7.1 – 7.13), resuspend the washed beads in the appropriate volume of 10 mM Tris-HCl (pH 8.0) as outlined in Step 7.14, and store the reactions at 4 °C for up to 24 hours.

DNA solutions containing beads must not be frozen, and beads must not be stored dry, as this is likely to damage the beads and result in sample loss. To resume the library construction process, centrifuge the reaction vessels briefly to recover any condensate, and add the remaining components required for the next enzymatic reaction in the protocol (see Tables 4B and 5B on p. 10). If the protocol was paused after the first post-ligation cleanup, continue directly with the second post-

Safe stopping points (continued)

Adapter-ligated DNA that has been completely cleaned up or size-selected can be stored at 4 °C for one week, or at -20 °C for at least one month before amplification, target enrichment, and/or sequencing. To avoid degradation, always store DNA in a buffered solution (10 mM Tris-HCl, pH 8.0) and minimize the number of freeze-thaw cycles.

Paramagnetic SPRI® beads and reaction cleanups

- Cleanups should be performed in a timely manner to ensure that enzyme reactions do not proceed beyond optimal incubation times.
- This protocol has been validated using Agencourt® AMPure® XP reagent (Beckman Coulter, part number A63880, A63881, or A63882). Solutions and conditions for DNA binding and size selection may differ if other beads are used.
- Observe all the manufacturer's storage and handling recommendations for AMPure® XP reagent.
- Beads will settle gradually; always ensure that they are fully resuspended before aspirating AMPure® XP reagent.
- The incubation times provided for reaction cleanups and size selection are guidelines only, and should be modified/optimized according to your current protocols, previous experience, and specific equipment and samples in order to maximize library construction efficiency and throughput.
- The time required to completely capture magnetic beads varies according to the reaction vessel and magnet used. It is important to not 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. Where possible, use a wash volume that is equal to the volume of sample plus AMPure® XP reagent or PEG/NaCl SPRI® Solution.
- It is important to remove all ethanol before proceeding with subsequent reactions. However, over-drying of beads may make them difficult to resuspend, and may result in a dramatic loss of DNA. With optimized pipetting, 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). Elution of DNA in PCR-grade water is not recommended, as DNA is unstable in unbuffered solutions.

Input DNA and fragmentation

- This protocol has been validated for library construction from 100 ng – 5 µg of appropriately fragmented, double-stranded DNA. However, libraries can be prepared from lower input amounts if the sample represents sufficient copies to ensure the requisite coverage and complexity in the final library. Successful library construction has been achieved from <100 pg of ChIP DNA, low nanogram quantities of cDNA or microbial DNA, and 1 – 10 ng of high-quality human or mouse genomic DNA.
- The above typically refers to the input into the end repair reaction. If input DNA is quantified before fragmentation, and/or fragmented DNA is subjected to cleanup or size selection prior to end repair, the actual input into library construction may be significantly lower. This should be taken into account when evaluating the efficiency of the process and/or during optimization of library amplification cycle number.
- The proportion of fragmented DNA that is successfully converted to adapter-ligated molecules decreases as input is reduced. When starting library construction (end repair) with >100 ng fragmented DNA, 15 – 40% of input DNA is typically recovered as adapter-ligated molecules, whereas the recovery typically ranges from 0.5 to 15% for libraries constructed from 100 pg – 100 ng DNA. These figures apply to high quality DNA and can be significantly lower for DNA of lower quality, e.g. FFPE samples. Workflows that contain additional SPRI® cleanups or size selection prior to library amplification are likely to result in a lower yield of adapter-ligated molecules.
- Solutions containing high concentrations of EDTA and strong buffers may negatively affect the end repair reaction, and should be avoided. If fragmented DNA will not be processed (i.e. subjected to cleanup or size selection) prior to end repair, DNA should be fragmented in 10 mM Tris-HCl (pH 8.0 or 8.5) with 0.1 mM EDTA. Fragmentation in water is not recommended.
- In some circumstances it may be convenient to fragment input DNA in 1X KAPA End Repair Buffer, in which case the end repair reaction setup should be adjusted accordingly. Please contact **support@kapabiosystems.com** for more information.

Cleanups after end repair and A-tailing

- This protocol provides for 1.7X – 1.8X cleanups after end repair and A-tailing. This ratio of PEG/NaCl SPRI® Solution to sample volume will retain most DNA fragments larger than ~75 bp. If you wish to retain very small DNA fragments, the PEG/NaCl SPRI® Solution to sample ratio can be increased to 2X – 3X for all cleanups prior to adapter ligation.

Cleanups after end repair and A-tailing (continued)

- If a >2X SPRI® bead cleanup is desired after end repair, the end repair reaction must be scaled down when performing library construction in standard PCR plates (maximum working volume of ~200 ul). Please contact **support@kapabiosystems.com** if your workflow or sample type requires modified SPRI® bead cleanups.

Adapter design and concentration

- This protocol has been validated using standard, indexed Illumina® TruSeq™ "forked" adapters, but the kit is compatible with other adapters of similar design.
- Adapter concentration affects ligation efficiency, as well as adapter and adapter-dimer carry-over in post-ligation cleanups. The optimal adapter concentration for your workflow represents a compromise between cost and the above factors. Your choice of post-ligation cleanup and size-selection options should be informed by your choice of adapter concentration. Please refer to **Important Parameters: Post-ligation cleanup** for more details.
- Ligation efficiency is robust for adapter:insert molar ratios ranging from 10:1 to 50:1. As a general guideline, we recommend an adapter:insert molar ratio of ~10:1, for libraries constructed from ≥100 ng fragmented DNA. This translates to different final adapter concentrations for libraries with different size distributions (see Table 1 below). An adapter:insert molar ratio >10:1 **may** be beneficial for libraries constructed from lower amounts of input DNA.
- While it is not necessary to adjust adapter concentrations to accommodate moderate sample-to-sample variations, we recommend using an adapter concentration that is appropriate for the range of input DNA concentrations.
- The best way to accommodate different adapter concentrations within a batch of samples processed together, is to vary the concentration of adapter stock solutions, and dispense a fixed volume (5 µl) of each adapter. The alternative – using a single stock solution, and dispensing variable volumes of adapter into ligation reactions – is not recommended for automated workflows.

Post-ligation cleanup

- It is important to remove excess unligated adapter and adapter-dimer molecules from the library prior to library amplification or cluster generation.
- While a single SPRI® bead cleanup removes most unligated adapter and adapter-dimer, a second SPRI® bead cleanup is recommended to eliminate any remaining adapter species from the library. The amount of adapter and adapter-dimer carried through the first cleanup is dependent on the adapter concentration in the ligation reaction.
- If size selection is carried out between adapter ligation and library amplification (or clustering), a single post-ligation cleanup with SPRI® beads (1X) is usually sufficient prior to size selection. If no post-ligation size selection is carried out, two consecutive 1X SPRI® bead cleanups are recommended.
- The volume in which washed beads are resuspended after the post-ligation cleanup(s) should be adjusted to suit your chosen workflow:
  - If proceeding directly to library amplification, determine an appropriate final volume in which to elute the library DNA, keeping in mind that you may wish to divert and/or reserve some of this library material for archiving and/or QC purposes. Since an optimized 50 µl library amplification reaction should yield ~1 µg of DNA, and can accommodate a maximum of 20 µl template DNA, an elution volume of 22 – 32 µl is recommended.
  - If proceeding with size selection, elute the library DNA in an appropriate volume according to the size selection method of choice. For the dual-SPRI® size selection procedure described here, beads have to be resuspended in a final elution volume of 100 µl.

Table 1. Recommended adapter concentrations.

Insert DNA per 50 µl end repair reaction	Recommended adapter concentration for DNA sheared to an average size of					
	175 bp		350 bp		500 bp	
	Stock	Final	Stock	Final	Stock	Final
3 – 5 µg	60 µM	6 µM	30 µM	3 µM	21 µM	2.1 µM
1 µg	20 µM	2 µM	10 µM	1 µM	7 µM	0.7 µM
500 ng	10 µM	1 µM	5 µM	500 nM	3.5 µM	350 nM
100 ng	2 µM	200 nM	1 µM	100 nM	700 nM	70 nM

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