KAPABIOSYSTEMS

Technical Data Sheet

KAPA Library Preparation Kit

Illumina® Platforms

KR0410 – v4.16

This Technical Data Sheet provides product information and a detailed protocol for KAPA Library Preparation for Illumina platforms.

Contents

Kapa/*Roche* Kit Codes and Components		
KK8200 *07961812001* 10 reactions	End Repair Enzyme Mix	50 µL
	End Repair Buffer with dNTPs (10X)	100 µL
	A-Tailing Enzyme	30 µL
	A-Tailing Buffer (10X)	50 µL
	DNA Ligase	50 µL
	Ligation Buffer (5X)	100 µL
	KAPA HiFi HotStart ReadyMix (2X)	250 µL
KK8201 *07961839001* 50 reactions	End Repair Enzyme Mix	250 µL
	End Repair Buffer with dNTPs (10X)	500 µL
	A-Tailing Enzyme	150 µL
	A-Tailing Buffer (10X)	250 µL
	DNA Ligase	250 µL
	Ligation Buffer (5X)	500 µL
	KAPA HiFi HotStart ReadyMix (2X)	1250 µL

Quick Notes

- This kit contains all the reagents for library construction and high-efficiency and low-bias library amplification except for adapters, beads and amplification primers. KAPA Pure Beads (KK8000, KK8001, KK8002), Library Amplification Primer Mix (KK2623), and KAPA Adapters are sold separately.
- Reaction components should be mixed fresh and used on the same day.
- Bead-based cleanups with KAPA Pure Beads are recommended for reaction cleanup steps between enzymatic reactions.
- The optimal cycling number for library amplification is determined by the volume and concentration of adapter-ligated library DNA added to each enrichment PCR reaction.
- For custom primers, a gradient PCR is recommended to optimize annealing temperature. For more information, please contact Technical Support at **kapabiosystems.com/support**.

Product Description

The KAPA Library Preparation Kit provides all of the enzymes and reaction buffers required for constructing libraries from fragmented dsDNA via the following steps:

1. end repair: Produce blunt-ended, 5'-phosphorylated fragments;
2. A-tailing: Add dAMP to the 3'-ends of the dsDNA library fragments;
3. adapter ligation: Ligate dsDNA adapters with 3'-dTMP overhangs to library fragments;
4. library amplification: PCR amplification of library fragments carrying appropriate adapter sequences on both ends.

Reaction buffers are supplied in convenient, concentrated "master mix" formats comprising all of the required reaction components except oligonucleotide adapters and PCR primers. Similarly, a single enzyme mixture is provided for each step of the library construction process, reducing the number of pipetting steps.

In order to maximize sequence coverage uniformity, it is critical to minimize library amplification bias. KAPA HiFi DNA Polymerase is designed for low-bias, high-fidelity PCR, and is the reagent of choice for NGS library amplification.[1,2,3,4] KAPA Library Preparation Kits include KAPA HiFi HotStart ReadyMix (2X), a ready-to-use PCR mix comprising all the components for library amplification—except primers and template.

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

This kit is primarily intended for the construction of genomic, paired-end, and paired-end multiplex (indexed/barcoded) Illumina DNA libraries, but may be used for other applications requiring efficient end-repair, A-tailing, ligation, and/or library amplification steps.

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 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

Always ensure that components have been fully thawed and thoroughly mixed before use. Keep all reaction components and master mixes on ice whenever possible during handling. The KAPA HiFi HotStart Ready Mix (2X) contains isostabilizers and may not freeze solidly, even when stored at -15°C to -25°C. Nevertheless, always ensure that the KAPA HiFi HotStart Ready Mix is fully thawed and has been vortexed before use. Please note that certain components in KAPA Library Preparation Kits (e.g., End Repair Mix, DNA Ligase, buffers containing dNTPs and/or ATP, etc.) are particularly sensitive to temperature and freeze-thaw cycles, and should be handled with special care.

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 Technical Support at **kapabiosystems.com/support** for more information.

Library Construction Protocol

Materials required but not supplied in this kit:

- Reaction tubes: Reactions may be assembled and processed in PCR plates, PCR tubes, or microcentrifuge tubes.
- Pipette tips: The use of high-quality filter-plugged tips is recommended to prevent contamination of reagents and library samples.
- Reaction cleanup: The use of either KAPA Pure Beads (KK8000, KK8001, KK8002) or Agencourt® AMPure® XP (Beckman Coulter®) is recommended.
- Adapters: KAPA Adapters are recommended, however, the kit is also compatible with non-indexed, single-indexed, and dual-indexed adapters for Illumina sequencing.
- Library amplification primers: Library Amplification Primer Mix (10X) (KK2623) is recommended for the amplification of libraries for Illumina sequencing as the primers contain the same sequences that are used for flowcell hybridization.

1. End Repair

1.1 Assemble each end repair reaction in a tube or well of a PCR plate as follows:

Component	Volume
Water to 100 µL	X µL
End Repair Buffer (10X)	10 µL
End repair enzyme mix	5 µL
Sheared dsDNA (1 – 5 µg)	1 – 85 µL
Total volume:	**100 µL**

1.2 Incubate for 30 min at 20°C.

1.3 Proceed immediately to **End Repair Cleanup** (step 2).

2. End Repair Cleanup

2.1 Ensure that KAPA Pure Beads have been equilibrated to room temperature and that the beads are fully resuspended before proceeding.

2.2 In the same plate/tube(s), perform a 1.6X bead-based cleanup by combining the following:

Component	Volume
End repair reaction	100 µL
KAPA Pure Beads	160 µL
Total volume:	**260 µL**

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

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

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

2.6 Carefully remove and discard the supernatant.

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

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

2.9 Carefully remove and discard the ethanol.

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

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

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

2.13 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.***

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

2.15 Thoroughly resuspend the beads in 32.5 µL elution buffer (10 mM Tris-HCl, pH 8.0 – 8.5).

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

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

2.18 Transfer the clear supernatant to a new plate/tube(s) and proceed with **A-tailing** (step 3).

SAFE STOPPING POINT

If you are not proceeding to **A-tailing** (step 3) immediately, the protocol can be safely stopped here. Store at -20ºC for ≤7 days.

3. A-tailing

3.1 In the plate/tube(s) containing end-repaired DNA, assemble the A-tailing reaction as follows::

Component	Volume
End-repaired DNA	30 µL
Water	12 µL
A-tailing Buffer (10X)	5 µL
A-tailing Enzyme	3 µL
Total volume:	**50 µL**

3.2 Incubate for 30 min at 30°C.

3.3 Proceed immediately to **A-tailing Cleanup** (step 4).

4. A-tailing Cleanup

4.1 Ensure that KAPA Pure Beads have been equilibrated to room temperature and that the beads are fully resuspended before proceeding.

4.2 In the same plate/tube(s), perform a 1.8X bead-based cleanup by combining the following:

Component	Volume
A-tailing reaction	50 µL
KAPA Pure Beads	90 µL
Total volume:	**140 µL**

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

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

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

4.6 Carefully remove and discard the supernatant.

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

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

4.9 Carefully remove and discard the ethanol.

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

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

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

4.13 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.***

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

4.15 Thoroughly resuspend the beads in 32.5 µL elution buffer (10 mM Tris-HCl, pH 8.0 – 8.5).

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

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

4.18 Transfer the clear supernatant to a new plate/tube(s) and proceed with **Adapter Ligation** (step 5).

SAFE STOPPING POINT

If you are not proceeding to **Adapter Ligation** (step 5) immediately, the protocol can be safely stopped here. Store at -20ºC for ≤7 days.

5. Adapter Ligation

KAPA Adapters (supplied separately) are recommended for use with the KAPA Library Preparation Kit. However, the kit is also compatible with non-indexed, single-indexed, and dual-indexed adapters that are routinely used in Illumina TruSeq®, Roche® NimbleGen™ SeqCap™ EZ, Agilent® SureSelect, and other similar library construction and target capture workflows. Custom adapters that are of a similar design and are compatible with TA-ligation of dsDNA may also be used, remembering that custom adapter designs may impact library construction efficiency.

5.1 In the plate/tube(s) containing the A-tailed DNA, assemble the adapter ligation reaction as follows:

Component	Volume
A-tailed DNA	30 µL
Adapter (30 µM)	5 µL
Ligation Buffer (5X)*	10 µL
DNA Ligase*	5 µL
Total volume:	**50 µL**

*The buffer and ligase enzyme should preferably be premixed and added in a single pipetting step. Premixes are stable for ≤24 hrs at room temperature, for ≤3 days at 4°C, and for ≤4 weeks at -20°C.

5.2 Incubate for 15 min at 20°C.

5.3 Proceed immediately to **1st Post-ligation Cleanup** (step 6).

6. 1st Post-ligation Cleanup

Depending on requirements and chosen workflow, either one post-ligation cleanup followed by size-selection or two post-ligation cleanups may be performed.

6.1 Ensure that KAPA Pure Beads have been equilibrated to room temperature and that the beads are fully resuspended before proceeding.

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

Component	Volume
Adapter ligation reaction	50 µL
KAPA Pure Beads	50 µL
Total volume:	**100 µL**

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

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

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

6.6 Carefully remove and discard the supernatant.

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

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

6.9 Carefully remove and discard the ethanol.

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

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

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

6.13 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.***

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

6.15 Thoroughly resuspend the beads in an appropriate volume of elution buffer (10 mM Tris-HCl, pH 8.0 – 8.5).

Recommended volumes are as follows:

- If proceeding to **2nd Post-ligation Cleanup** (step 7), resuspend the beads in 50 µL.
- If proceeding to **Size Selection** (step 8), resuspend the beads in 100 µL, and omit **2nd Post-ligation Cleanup** (step 7).
- For a **safe stopping point**, resuspend the beads in either 50 µL or 100 µL (as required), and store at 4°C for ≤24 hrs.

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

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

6.18 Transfer the clear supernatant to a new plate/tube(s) and proceed with either **2nd Post-ligation Cleanup** (step 7) or **Size Selection** (step 8).

7. 2nd Post-ligation Cleanup

7.1 Ensure that KAPA Pure Beads have been equilibrated to room temperature and that the beads are fully resuspended before proceeding.

7.2 In the plate/tube(s) containing the adapter ligated library from the first cleanup reaction, perform a 1X bead-based cleanup by combining the following:

Component	Volume
Purified adapter ligated library DNA	50 µL
KAPA Pure Beads	50 µL
Total volume:	**100 µL**

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

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

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

7.6 Carefully remove and discard the supernatant.

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

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

7.9 Carefully remove and discard the ethanol.

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

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

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

7.13 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.***

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

7.15 Thoroughly resuspend the beads in 32.5 µL elution buffer (10 mM Tris-HCl, pH 8.0 – 8.5).

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

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

7.18 Transfer the clear supernatant to a new plate/tube(s).

7.19 Proceed to **Library Amplification** (step 9). Store purified, adapter-ligated libraries at 4°C for 1 – 2 weeks or at -20°C for ≤1 month.

8. Size Selection

Size selection requirements vary widely for different sequencing applications. If required, any commonly used bead- or gel-based size selection technique may be integrated in the KAPA Library Preparation Kit workflow.

The double-sided size selection (0.6X - 0.8X) procedure described here is designed for selection of adapter-ligated fragments approximately 250 – 450 bp in length. If you wish to select a different range of fragment lengths, please consult the **KAPA NGS Library Preparation Technical Guide** or contact Technical Support at **kapabiosystems.com/support**.

8.1 Perform the first size cut (0.6X) by combining the following:

Component	Volume
Purified, adapter-ligated DNA	100 µL
KAPA Pure Beads	60 µL
Total volume:	**160 µL**

8.2 Thoroughly resuspend the beads by vortexing and/or pipetting up and down multiple times.

8.3 Incubate the plate/tube(s) at room temperature for 5 – 15 min to bind library fragments larger than ~450 bp to the beads.

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

8.5 Carefully transfer 155 µL of supernatant containing library fragments smaller than ~450 bp to a new plate/tube(s). ***It is critical that no beads are transferred with the supernatant.***

8.6 Discard the plate/tube(s) with beads, to which library fragments larger than ~450 bp are bound.

8.7 Perform the second size cut (0.8X) by combining the following:

Component	Volume
Supernatant from first cut	155 µL
KAPA Pure Beads	20 µL
Total volume:	**175 µL**

8.8 Thoroughly resuspend the beads by vortexing and/or pipetting up and down multiple times.

8.9 Incubate the plate/tube(s) at room temperature for 5 – 15 min to bind library fragments larger than ~250 bp to the beads.

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

8.11 Carefully remove and discard the supernatant, which contains library fragments smaller than ~250 bp.

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

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

8.14 Carefully remove and discard the ethanol.

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

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

8.17 Carefully remove and discard.

8.18 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.***

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

8.20 Thoroughly resuspend the beads in 25 µL of elution buffer (10 mM Tris-HCl, pH 8.0 – 8.5).

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

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

8.23 Transfer the clear supernatant containing size-selected DNA to a new plate/tube(s) and proceed with **Library Amplification** (step 9). Store size-selected, purified, adapter-ligated libraries at 4ºC for 1 – 2 weeks or at -20ºC.

9. Library Amplification

The Library Amplification Primer Mix (10X) (KK2623), sold separately, is designed to eliminate or delay primer depletion during library amplification reactions performed with KAPA HiFi HotStart ReadyMix. 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 described below. 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 **kapabiosystems.com/support**.

9.1 Assemble each library amplification reaction in the plate/tube(s) to which purified (size selected) adapter-ligated library was transferred, or in a new PCR plate/set of tubes (if only a portion of the library is amplified). Reactions are comprised as follows:

Component	Volume
KAPA HiFi HotStart ReadyMix (2X)	25 µL
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.

9.2 Mix thoroughly and centrifuge briefly.

9.3 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 (10 – 18 cycles)
Annealing*	60°C	30 sec	
Extension	72°C	30 sec	
Final extension	72°C	1 min	1
HOLD	4°C	∞	1

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

*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. Typically, this is in the 10 – 18 cycle range but may require optimization.

9.4 Proceed directly to **Post-amplification Cleanup** (step 10).

10. Post-amplification Cleanup

10.1 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**

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

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

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

10.5 Carefully remove and discard the supernatant.

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

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

10.8 Carefully remove and discard the ethanol.

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

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

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

10.12 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.***

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

10.14 Thoroughly resuspend the beads in an appropriate volume of elution buffer (10 mM Tris-HCl, pH 8.0 – 8.5) or PCR-grade water. ***Always use PCR-grade water if proceeding to target capture.***

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

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

10.17 Transfer the clear supernatant to a new plate/tube(s) and proceed with **Evaluating the Success of Library Construction** (step 11). Store purified, amplified libraries at 4°C for 1 – 2 weeks, or at -20°C.

11. Evaluating the Success of Library Construction

The size distribution of pre-capture or final libraries should be confirmed with an electrophoretic method. A LabChip® GX, GXII, or GX Touch (PerkinElmer), Bioanalyzer or TapeStation (Agilent Technologies), Fragment Analyzer™ (Advanced Analytical) or similar instrument is recommended over conventional gels.

KAPA Library Quantification Kits (KK4824, KK4835, KK4844, KK4854, KK4873) are recommended for qPCR-based quantification of libraries generated using the KAPA Library Preparation Kit. These kits employ primers based on the Illumina flow cell oligos, and provide the only reliable means for quantifying libraries at different stages of the workflow. The KAPA Library Quantification Kit only quantifies those molecules with two adapters in the correct configuration for cluster amplification and sequencing, and measurements are not affected by library over-amplification.

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