KAPABIOSYSTEMS

Technical Data Sheet

KAPA Single-Indexed Adapter Kit

Illumina® Platforms

KR1317 – v3.17

This Technical Data Sheet provides product information and guidelines for use of KAPA Single-Indexed Adapter Kits for Illumina platforms.

This document applies to KAPA Single-Indexed Adapter Kits A and B (30 µM) for Illumina platforms (08005699001, 08005702001 and 08005729001), KAPA Single-Indexed Adapter Kits A and B (1.5 µM) for Illumina platforms (08005770001, 08005788001 and 08005796001), KAPA Single-Indexed Adapter Kits 1 and 2 (30 µM) for Illumina platforms (08005737001 and 08005745001), and KAPA Single-Indexed Adapter Kits 1 and 2 (1.5 µM) for Illumina platforms (08005800001 and 08005818001).

Contents

KAPA/*Roche* Kit Codes and Components		
KK8700 *08005699001*	KAPA Single-Indexed Adapter Set A + B (30 µM) Adapter Dilution Buffer	24 x 40 µL 4 x 5 mL
KK8701 *08005702001*	KAPA Single-Indexed Adapter Set A (30 µM) Adapter Dilution Buffer	12 x 40 µL 2 x 5 mL
KK8702 *08005729001*	KAPA Single-Indexed Adapter Set B (30 µM) Adapter Dilution Buffer	12 x 40 µL 2 x 5 mL
KK8710 *08005770001*	KAPA Single-Indexed Adapter Set A + B (1.5 µM) Adapter Dilution Buffer	24 x 40 µL 4 x 5 mL
KK8711 *08005788001*	KAPA Single-Indexed Adapter Set A (1.5 µM) Adapter Dilution Buffer	12 x 40 µL 2 x 5 mL
KK8712 *08005796001*	KAPA Single-Indexed Adapter Set B (1.5 µM) Adapter Dilution Buffer	12 x 40 µL 2 x 5 mL
KK8703 *08005737001*	KAPA Single-Indexed Adapter Set 1 (30 µM) Adapter Dilution Buffer	4 x 40 µL 1 x 5 mL
KK8704 *08005745001*	KAPA Single-Indexed Adapter Set 2 (30 µM) Adapter Dilution Buffer	4 x 40 µL 1 x 5 mL
KK8713 *08005800001*	KAPA Single-Indexed Adapter Set 1 (1.5 µM) Adapter Dilution Buffer	4 x 40 µL 1 x 5 mL
KK8714 *08005818001*	KAPA Single-Indexed Adapter Set 2 (1.5 µM) Adapter Dilution Buffer	4 x 40 µL 1 x 5 mL

Adapter Set A contains indices 2, 4, 5, 6, 7, 12, 13, 14, 15, 16, 18, and 19.
Adapter Set B contains indices 1, 3, 8, 9, 10, 11, 20, 21, 22, 23, 25, and 27.
Adapter Set 1 contains indices 5, 6, 12, and 19.
Adapter Set 2 contains indices 2, 4, 7, and 16.

Quick Notes

- KAPA Single-Indexed Adapter Kits for Illumina platforms contain 40 µL of each indexed adapter, supplied at a concentration of either 30 µM (high concentration) or 1.5 µM (low concentration). Please refer to **Table 1** for guidelines on which kit to use for different inputs and applications.
- The number of libraries that can be prepared with each adapter kit is dependent on the amount of input DNA, the average fragment size of the input DNA, and the kit used for library construction. Please refer to **Important Parameters** for guidelines on how to use KAPA Single-Indexed Adapters in combination with different KAPA library preparation kits.
- KAPA Single-Indexed Adapters are duplexed oligonucleotides and must not be exposed to temperatures above room temperature. Adapters must be diluted in the Adapter Dilution Buffer provided in the kit to avoid dissociation and ensure optimal performance.
- Employ best laboratory practices to avoid cross-contamination of indexed adapters.
- To ensure equal read distributions in multiplexed sequencing applications, libraries must be carefully quantified and/or normalized prior to pooling for capture or cluster generation. qPCR-based quantification with the KAPA Library Quantification Kit constitutes the most accurate and reproducible method for the quantification of sequenceable molecules. This is particularly true for PCR-free workflows.

Product Description

KAPA Single-Indexed Adapter Kits for Illumina platforms are designed for use with KAPA DNA and RNA library preparation kits to construct libraries for sequencing on an Illumina sequencer. Kits contain a set of 12 or 24 adapters, each with a single, 6-nucleotide index (barcode) for multiplexed sequencing applications.

Adapters are available in high (30 µM) or low (1.5 µM) concentration kits. Please refer to **Table 1** in **Important Parameters: Compatibility with KAPA Library Preparation Kits** (p. 3) for guidelines on which concentration to use for different library types.

The sequences of the sequencing indices (barcodes) included in KAPA Single-Indexed Adapter Kits are given in **Important Parameters: Index Sequences and Pooling Guidelines** (p. 6). The highly dissimilar nature of indices minimizes the possibility of inaccurate index calling.

Adapter Dilution Buffer (10 mM Tris-HCl, (pH 8.0 – 8.5), 10 mM NaCl, 1 mM EDTA) is provided with the kit to ensure optimal performance when adapters have to be diluted.

Product Applications

KAPA Single-Indexed Adapters are used to uniquely label sequencing libraries generated from individual biological samples. This allows for the pooling of libraries prior to target capture or cluster generation, to enable multiplexed sequencing—which simplifies sample preparation and reduces the cost of next-generation sequencing for a wide range of applications.

Primary applications for the use of KAPA Single-Indexed Adapter kits for Illumina platforms include:

- whole exome or targeted sequencing, using Roche SeqCap EZ or IDT xGen Lockdown Probes or other hybridization capture systems, in combination with the appropriate blockers
- RNA-seq
- ChIP-seq
- other direct sequencing applications, e.g., microbial whole-genome sequencing on compatible platforms.

NOTE: KAPA Single-Indexed Adapter Kits are not validated for sequencing on the Illumina HiSeq X instrument. In addition, KAPA Single-Indexed Adapters are not methylated, and can therefore not be used for methyl-seq applications.

Product Specifications

Shipping and Storage

KAPA Single-Indexed Adapter Kits are shipped on dry ice or ice packs, depending on the destination country. Upon receipt, immediately store the adapters at -15°C to -25°C in a constant-temperature freezer. Adapters must not be exposed to temperatures above room temperature. When stored under these conditions and handled correctly, the adapters will retain full functionality until the expiry date indicated on the kit label. The Adapter Dilution Buffer may be stored at 2°C to 8°C for short-term use, but -15°C to -25°C is recommended for long-term storage.

Handling

Always ensure that adapters have been fully thawed, kept on ice and/or actively cooled, and are thoroughly mixed before use.

Quality Control

KAPA Single-Indexed Adapters are subject to stringent functional and barcode cross-contamination quality control. KAPA Adapter Dilution Buffer is free of detectable contaminating exo- and endonuclease activities, and meet strict requirements with respect to DNA contamination. For more information, please contact Technical Support at **sequencing.roche.com/support**.

Important Parameters

Best Practices

- KAPA Single-Indexed Adapters should be used on ice or in cooled reagent blocks, and must not be exposed to conditions above room temperature.
- Use the Adapter Dilution Buffer (10 mM Tris-HCl, (pH 8.0 – 8.5), 10 mM NaCl, 1 mM EDTA) provided in the kit to dilute KAPA Single-Indexed Adapters. Adapters diluted in any other buffer or in PCR-grade water may not support optimal library construction efficiency.
- Dilute only the amount of each adapter needed for same-day usage. Long-term storage and multiple cycles of freezing and thawing of diluted adapter stocks are not recommended.
- Employ best laboratory practices to avoid cross-contamination of adapters and/or the dilution buffer.
- Always use plastics that are certified to be free of DNAses, RNAses, and nucleases. Low DNA- and RNA-binding plastics are highly recommended, especially for low-input DNA and all RNA-Seq library construction applications.

Compatibility with KAPA Library Preparation Kits

KAPA Single-Indexed Adapter Kits for Illumina platforms are recommended for use with all KAPA library construction kits.

Table 1 indicates which adapter stock concentration (30 µM or 1.5 µM) is best suited for library construction depending on the input amount and the kit used for library preparation.

Table 1. Recommended adapter concentration by input

KAPA Library Preparation Kit*	30 µM	1.5 µM
KAPA HyperPlus and Hyper Prep Kits	≥5 ng – 1 µg	<5 ng
KAPA HTP or LTP "with-bead" Library Preparation Kits	≥100 ng**	≤100 ng**
KAPA Stranded RNA-Seq Kits with RiboErase (HMR)	Not recommended	All inputs and workflows
KAPA Stranded RNA-Seq and mRNA-Seq Kits	Not recommended	All inputs and workflows

*Please consult individual Technical Data Sheets for **RNA HyperPrep Kits** for input recommendations.

**For 100 ng input, the recommended adapter concentration depends on average insert size.

When selecting a KAPA Single-Indexed Adapter Kit for your application, please consider the following:

- Refer to the table corresponding to the KAPA library preparation kit that you will be using. Recommended adapter stock concentrations for libraries constructed from different inputs and DNA fragment lengths with the kits listed above are included in Tables 2 – 6 on p. 4.
- Identify the recommended adapter stock concentration for your input and fragment length. If that concentration is >1.5 µM, use a high concentration (30 µM) KAPA Single-Indexed Adapter Kit. If that concentration is ≤1.5 µM, use a low concentration (1.5 µM) KAPA Single-Indexed Adapter Kit.
- If your input and specific fragment length is not listed in the table, follow the instructions in **Adapter Concentration Calculations** (p. 5) to calculate the appropriate adapter stock concentration for your experiment, or contact **sequencing.roche.com/support** to obtain a calculator designed for this purpose.
- For each batch of libraries to be constructed, prepare an appropriate volume of diluted adapter using the Adapter Dilution Buffer provided in the kit, remembering that:
  - Standard protocols call for 5 µL of appropriately diluted adapter stock per library.
  - If an adapter stock concentration >30 µM is required, the volume of water in the ligation reaction may be reduced and the volume of adapter increased to the same extent, up to a total of 10 µL adapter per reaction.
  - Diluted adapters should be freshly prepared and must not be stored for long periods of time or subjected to repeated freezing and thawing.
  - An excess volume of each diluted adapter stock will be required to ensure accurate dispensing. The excess will be larger for automated vs. manual use.
  - All or some of the libraries in the batch may require a different index. Please refer to **Index Sequences and Pooling Guidelines** (p. 6) for recommendations on multiplexing.
  - The Technical Data Sheet included with your library preparation kit contains specific guidelines for the optimization of adapter concentration when using that particular kit for different applications.

Table 2. Recommended adapter concentrations for KAPA Hyper Prep and HyperPlus Kits[1]

Fragmented DNA per 60 µL ER & AT reaction	Adapter stock concentration	Adapter:insert molar ratio	Fragmented DNA per 60 µL ER & AT reaction	Adapter stock concentration	Adapter:insert molar ratio
1 µg	15 µM	10:1	25 ng	7.5 µM	200:1
500 ng	15 µM	20:1	10 ng	3 µM	200:1
250 ng	15 µM	40:1	5 ng	1.5 µM	200:1
100 ng	15 µM	100:1	2.5 ng	750 nM	200:1
50 ng	15 µM	200:1	1 ng	300 nM	200:1

[1]Adapter:insert molar ratio calculations are based on a mode DNA fragment length of 200 bp, and will be higher for longer DNA fragments, or slightly lower for DNA fragmented to a mode size <200 bp. The lower adapter:insert molar ratios recommended for inputs >100 ng represent a fair compromise between library construction efficiency and cost; higher library yields will be achieved if a higher adapter concentration is used.

Table 3. Recommended adapter concentrations for KAPA HTP and LTP Library Preparation Kits[1]

Insert DNA per 70 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
10 ng	200 nM	20 nM	100 nM	10 nM	70 nM	7 nM

[1]Adapter concentrations are based on a ~10:1 adapter:insert ratio. For values not listed here, please refer to the calculation in **Important Parameters: Adapter Concentration Calculations** or visit **kapabiosystems.com/support** to obtain a calculator designed for this purpose.

Table 4. Recommended adapter concentrations for KAPA RNA HyperPrep Kits

KAPA RNA HyperPrep			KAPA RNA HyperPrep with RiboErase			KAPA mRNA HyperPrep	
Input DNA (all fragment lengths)	Starting material quality	Adapter stock concentration	Input DNA (all fragment lengths)	Starting material quality	Adapter stock concentration	Input DNA (all fragment lengths)	Adapter stock concentration
1 – 49 ng	Partially degraded or FFPE-derived	1.5 µM	25 – 499 ng	Partially degraded or FFPE-derived	1.5 µM	50 – 499 ng	1.5 µM
1 – 49 ng	High quality	1.5 µM	25 – 499 ng	High quality	1.5 µM	500 – 1000 ng	7 µM
50 – 100 ng	Partially degraded or FFPE-derived	1.5 µM	500 – 1000 ng	Partially degraded or FFPE-derived	1.5 µM		
50 – 100 ng	High quality	15 µM	500 – 1000 ng	High quality	7 µM		

Table 5. Recommended adapter concentrations for KAPA Stranded RNA-Seq Kits with RiboErase (HMR)

Input RNA	Adapter stock concentration	Final adapter concentration
501 – 1000 ng	280 nM	20 nM
251 – 500 ng	210 nM	15 nM
100 – 250 ng	140 nM	10 nM

Table 6. Recommended adapter concentrations for KAPA Stranded RNA and mRNA-Seq Library Preparation Kits

Input RNA		Adapter stock concentration	Final adapter concentration
RNA	mRNA Workflow		
201 – 400 ng	2001 – 4000 ng	1400 nM	100 nM
51 – 200 ng	501 – 2000 ng	700 nM	50 nM
10 – 50 ng	251 – 500 ng	350 nM	25 nM
	100 – 250 ng	140 nM	10 nM

Additional Notes on Adapter Concentration

- Adapter concentration affects ligation efficiency as well as adapter and adapter-dimer carry-over in post-ligation cleanups. A molar excess of adapter is required to ensure optimal ligation efficiency. Low adapter:insert molar ratios (approaching 2:1) result in a significant proportion of insert molecules with an adapter ligated to only one end, leading to library construction failure.
- Adapter:insert molar ratios in the range of 10:1 – 40:1 are recommended for KAPA HTP and LTP Library Preparation Kits, whereas KAPA Hyper Prep and KAPA HyperPlus Kits are compatible with much higher ratios (≥100:1). Very high adapter:insert molar ratios (200:1 – 1000:1) are beneficial for low-input library construction with KAPA Hyper Prep and KAPA HyperPlus Kits.
- While it is not necessary to adjust adapter concentrations to accommodate moderate sample-to-sample variations, an adapter concentration that is appropriate for the range of input DNA concentrations is highly recommended.
- 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 (e.g., 5 µL) of each adapter. The alternative—using a single stock solution and dispensing variable volumes of adapter into ligation reactions—is not recommended and is not compatible with higher throughput or automated workflows.
- Post-ligation cleanup and size selection strategies should be informed by your choice of adapter concentration. Please refer to **Important Parameters: Post-ligation Processing** for more details.
- Ultimately, the optimal adapter concentration for your specific workflow represents a compromise between ligation efficiency, the potential negative impact of adapter/adapter-dimer carry-over, and cost.

Adapter Concentration Calculations

- The below applies to DNA library construction. For RNA library construction, adapter stock concentrations are calculated based on input only.
- To calculate the optimal adapter stock concentration for DNA library construction, the amount of input DNA (in picomoles) must first be calculated. This is done with the following formula:

$$\text{Picomoles} = \frac{\text{mass of DNA (ng)}}{660} \times \frac{1000}{\text{median size (bp)}}$$

- Next, the picomole quantity of adapter required is calculated by multiplying the number of picomoles of input DNA by the desired adapter:insert ratio. Please refer to Tables 2 and 3 or the Technical Data Sheet included with your library preparation kit for optimal adapter:insert molar ratios for different applications.
- The picomole quantity of adapter required is subsequently divided by the volume of adapter used per reaction, to obtain the desired adapter stock concentration (in µM or picomoles/µL).

  For example, 200 ng of input DNA with a mode fragment size of 250 bp represents 1.21 picomoles of insert DNA. For a 10:1 adapter:insert ratio, 12.1 picomoles of adapter is required. Therefore, when using 5 µL of adapter stock per ligation reaction, an adapter stock concentration of 2.42 µM is required.
- Please visit **www.sequencing.roche.com** to obtain a calculator designed for the calculation of adapter:insert molar ratios and stock concentrations.

Post-ligation Processing

- It is important to remove excess unligated adapter and adapter-dimer molecules from Illumina libraries prior to library amplification or cluster generation. This is particularly important for libraries to be sequenced on Illumina platforms that employ patterned flow cells.
- Please follow the post-ligation cleanup instructions provided in the Technical Data Sheet for your library preparation kit. While a single post-ligation cleanup with KAPA Pure Beads or Agencourt AMPure XP removes most unligated adapter and adapter-dimer (as recommended for KAPA Hyper Prep and HyperPlus protocols), a second cleanup or size selection step may be necessary 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 library construction chemistry and adapter concentration in the ligation reaction.
- If bead-based size selection is carried out after adapter ligation, a single post-ligation cleanup (with the appropriate bead to sample ratio; as per your library construction protocol) must first be performed. Ligation buffers contain high concentrations of PEG 6000, which will impact the length and distribution of library fragments recovered from post-ligation size selection.

Index Sequences and Pooling Guidelines

- Sequencing indices (barcodes) included in KAPA Single-Indexed Adapters are given in Table 7.
- For low-plexity pooling applications (up to 4-plex) on Illumina sequencing platforms, specific index combinations must be used. For 5- to 11-plex pools, any of the 4-plex options may be used in combination with any other available adapters. Detailed multiplexing guidelines are provided in Table 8.
- To ensure equal read distributions in multiplexed sequencing applications, libraries must be carefully quantified and/or normalized prior to pooling for capture or cluster generation. qPCR-based quantification with the KAPA Library Quantification Kit for Illumina® platforms constitutes the most accurate and reproducible method for the quantification of sequenceable molecules in an Illumina library, particularly for PCR-free workflows.

Table 7. Index sequences

KAPA Single-Indexed Adapter Set A

Index	Sequence*
2	CGATGT
4	TGACCA
5	ACAGTG
6	GCCAAT
7	CAGATC
12	CTTGTA
13	AGTCAACA
14	AGTTCCGT
15	ATGTCAGA
16	CCGTCCCG
18	GTCCGCAC
19	GTGAAACG

KAPA Single-Indexed Adapter Set B

Index	Sequence*
1	ATCACG
3	TTAGGC
8	ACTTGA
9	GATCAG
10	TAGCTT
11	GGCTAC
20	GTGGCCTT
21	GTTTCGGA
22	CGTACGTA
23	GAGTGGAT
25	ACTGATAT
27	ATTCCTTT

*The barcode sequence in adapters 13 – 27 contains an additional two nucleotides (underlined) which may be incorporated into the sequencing index.

Table 8. Detailed multiplexing guidelines

Plexity	Option	Set A	Set B
2	1	Adapter 6 and 12	Not recommended
	2	Adapter 5 and 19	
3	1	Adapter 2, 7, and 19	Adapter 1, 10, and 20
	2	Adapter 5, 6, and 15	Adapter 3, 9, and 25
	3	Any of the 2-plex options with any other available adapter	Adapter 8, 11, and 22
4	1	Adapter 5, 6, 12, and 19	Adapter 1, 8, 10, and 11
	2	Adapter 2, 4, 7, and 16	Adapter 3, 9, 22, and 27
	3	Any of the 3-plex options with any other available adapter	Any of the 3-plex options with any other available adapter

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