KAPABIOSYSTEMS

Technical Data Sheet

KAPA Unique Dual-Indexed Adapter Kit
Illumina® Platforms

KR1736 – v6.24

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

This document applies to the KAPA Unique Dual-Indexed Adapter Kit (08861919702), KAPA Unique Dual-Indexed Adapter Plate (08861862001) and the standalone KAPA Adapter Dilution Buffer (08278539001).

Contents

KAPA/*Roche* Kit Codes and Components		
KK8727 *08861919702**	KAPA Unique Dual-Indexed Adapter Plate (20 µL/well)**	96 x 15 µM
	KAPA Adapter Sealing Foils (pierceable)	3 foils
	KAPA Adapter Dilution Buffer	25 mL
KK8726 *08861862001*	KAPA Unique Dual-Indexed Adapter Plate (20 µL/well)**	96 x 15 µM
	KAPA Adapter Sealing Foils (pierceable)	3 foils
KK8721 *08278539001*	KAPA Adapter Dilution Buffer	25 mL

*KK8727 (08861919702) = KK8726 (08861862001) + KK8721 (08278539001)
**Sufficient overage is included for use on automated liquid handlers

Quick Notes

- KAPA Unique Dual-Indexed Adapters are full-length adapters used during ligation-based library construction for sequencing on Illumina® instruments. Each of the 96 adapters contains a unique combination of two 8-nucleotide sequencing indexes (barcodes), which are never repeated throughout the set. **The barcodes are exclusive to Roche and differ from those provided by other suppliers.**
- The KAPA Unique Dual-Indexed Adapter Kit contains 20 µL of each indexed adapter, supplied at a concentration of 15 µM in a 96-well plate.
- The number of libraries that can be prepared with each KAPA Unique Dual-Indexed 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. With no dilution, four libraries can be prepared from each of the 96 indexed adapters using manual methods.
- KAPA Unique Dual-Indexed Adapters are duplexed oligonucleotides and must not be exposed to temperatures above room temperature.
- If required, adapters must be diluted in the KAPA 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. Do not vortex the adapter plate. Centrifuge the adapter plate then **carefully** remove the foil cover. Three additional adhesive, pierceable foils are provided in the kit.
- To ensure equal distribution of sequencing reads in multiplexed 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 is recommended for the quantification of sequenceable molecules, particularly for PCR-free workflows.

Product Description

KAPA Unique-Dual Indexed Adapters comprise a set of 96 full-length adapters used during ligation-based library construction for sequencing on Illumina® instruments. Each KAPA Unique Dual-Indexed Adapter contains two, non-redundant, 8-nucleotide indexes (sequencing barcodes) for multiplexed sequencing applications. These adapters are designed for use with KAPA DNA and RNA library preparation kits.

Each KAPA Unique Dual-Indexed Adapter has been manufactured by duplexing (hybridizing) two oligonucleotides. The backbones of these oligonucleotides are identical to those employed in Illumina TruSeq® adapters, however the sequences of the sample indexes included in KAPA Unique Dual-Indexed Adapters are exclusive to Roche, and differ from those employed by other suppliers. The 192 non-redundant indexes combined to create 96 Unique Dual-Indexed Adapters are listed in Table 2. KAPA Unique Dual-Indexed Adapters are designed and formulated to ensure high library construction efficiency and low adapter-dimer formation, and mitigate the technical challenges associated with index misalignment ("index hopping") on Illumina sequencers that employ patterned flow cells and exclusion amplification chemistry.[1]

KAPA 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 require further dilution.

[1]Illumina. Effects of Index Misassignment on Multiplexing and Downstream Analysis. 2017

Product Applications

KAPA Unique Dual-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 Unique Dual-Indexed Adapter Kit for Illumina platforms include:

- Human whole-genome sequencing, particularly PCR-free workflows
- whole-exome or targeted sequencing, using hybridization capture systems (in combination with compatible blockers)
- RNA-seq
- ChIP-seq
- other direct sequencing applications, e.g., microbial whole-genome sequencing on compatible platforms

NOTE: KAPA Unique Dual-Indexed Adapters are not methylated, and can therefore not be used for Methyl-Seq applications.

Product Specifications

Shipping and Storage

KAPA Unique Dual-Indexed Adapter Kits are shipped on dry ice or ice packs, depending on the destination country. Upon receipt:

- Immediately store the product at -15°C to -25°C in a constant-temperature freezer.
- Store the adapter plate in an upright orientation only.
- Do not expose adapters to temperatures above room temperature (~25°C) for extended periods.

The KAPA 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. When stored under these conditions and handled correctly, the adapters will retain full functionality until the expiry date indicated on the kit label.

Quality Control

KAPA Unique Dual-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 meets strict requirements with respect to DNA contamination.

Safety Information

Precautions

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material can vary, the operator must optimize pathogen inactivation and follow the appropriate measures according to local safety regulations.
- Do not eat, drink, or smoke in the laboratory area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats, and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online (eLabDoc) or upon request from the local Roche office.

For Research Use Only. Not for use in diagnostic procedures.

Important Parameters

Best Practices

- Always work with KAPA Unique Dual-Indexed Adapters on ice or in cooled reagent blocks, and avoid exposing the adapters to temperatures above room temperature (~25°C) for extended periods.
- 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.

Procedure for Handling KAPA Unique Dual-Indexed Adapter Plates

- **IMPORTANT!** The foil cover used for shipment of the product is not pierceable, only peelable. This is to ensure seal integrity is maintained and that plates do not leak during shipment. The three replacement foil seals provided in the kit are both pierceable and peelable.
- Remove the adapter plate from its packaging sleeve and thaw at room temperature or in a suitable cooled reagent block. Place on ice once completely thawed.
- Centrifuge the adapter plate at room temperature (e.g., for 1 minute at 280 x *g*) to ensure that all liquid is collected in the bottom of wells before the seal is removed.
  - **Do not vortex the adapter plate as it could result in cross-contamination of the Unique Dual-Indexed Adapters. Pipette mix individual adapters prior to use.**
- Upon first use, **carefully** remove the foil cover to avoid cross-contamination of the KAPA Unique Dual-Indexed Adapters. Discard the original foil cover. Do not reuse. If the adapters are to be used with an automated liquid handling system that requires a pierceable seal, cover the plate with one of the seals included in the kit.
- If using only a subset of KAPA Unique Dual-Indexed Adapters, partially remove the foil seal from the desired adapters by first using a sterile scalpel to make an incision in the foil. Be careful not to tear the foil unevenly. Do not reuse the partial seal.
- Remove the desired volume of each KAPA Unique Dual-Indexed Adapter as required for your experiment.
- If you are not using the entire contents of the KAPA Unique Dual-Indexed Adapter plate at this time, apply a new adhesive foil seal from one of the pierceable/peelable seals provided in the kit. Make sure that the foil is properly aligned and fully covers all 96 wells. Use a roller or other appropriate tool to ensure that the foil is evenly applied.
- Store the re-sealed KAPA Unique Dual-Indexed Adapter plate upright at -15°C to -25°C in a constant-temperature freezer for subsequent use.
- Three additional adhesive, pierceable and peelable foils are provided in the kit (4titude PCR Foil Seal, cat.no. 4ti-0550). If needed, additional replacement seals may be ordered, or any other suitable, pierceable/peelable seals from standard laboratory stocks may be used.

Additional Information pertaining to the use of KAPA Unique Dual-Indexed Adapter Plates on Automated platforms

- KAPA Unique Dual-Indexed Adapters are provided in fully-skirted, hard-shell plates (Bio-Rad, cat. no. HSP9901F). The exact dimensions of the plate are available upon request from Technical Support at sequencing.roche.com/support.
- Adapters are provided at a concentration of 15 µM. Each well contains 20 µL plus excess. Sufficient overage is included for use on automated liquid handlers
- **IMPORTANT!** The foil cover used for shipping is not pierceable, only peelable. This is to ensure seal integrity is maintained and that plates do not leak during shipment. If required by your method or system, **carefully** remove the foil cover and replace with a replacement foil (piercable and peelable) prior to using the adapter plate on automated liquid handling platforms.
- The adapter plate contains a linear barcode on the east and south side of the plate when viewed in the orientation depicted in Figure 1 on p. 6.
- Depending on the optimized low-volume pipetting settings for your method or platform, the number of libraries that can be prepared with each KAPA Unique Dual-Indexed Adapter will vary between two and four.

Compatibility with KAPA Library Preparation Kits

KAPA Unique Dual-Indexed Adapter Kits for Illumina platforms are designed for use in combination with the following library construction kits and workflows:

- KAPA HyperPrep Kits
- KAPA HyperPlus Kits
- KAPA EvoPlus V2 Kits
- KAPA EvoPrep Kits
- KAPA RNA HyperPrep Kits with RiboErase (HMR), or RiboErase (HMR) Globin
- KAPA RNA and mRNA HyperPrep Kits
- KAPA Stranded RNA-Seq Kit with RiboErase (HMR), or RiboErase (HMR) Globin
- KAPA Stranded RNA-Seq and mRNA-Seq Kits

KAPA Unique Dual-Indexed Adapter Working Concentrations

For most DNA applications, KAPA Unique Dual-Indexed Adapters will be used as supplied, i.e., at a working concentration of 15 µM. However, a single dilution will be required for low-input DNA and most RNA-Seq libraries.

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.

Please consult the Operator Manual of your specific KAPA library preparation kit for recommended adapter stock concentrations when constructing libraries from different inputs and fragment lengths; as well as for specific guidelines on how to optimize adapter concentration when using that particular kit for specific applications.

Adapter Concentration Calculations

- The calculation below applies to DNA library construction. For RNA library construction, adapter stock concentrations are calculated based on input only.
- To calculate the optimal working 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 the Operator Manual 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 2.4 µM is required.
- To obtain a calculator designed for the calculation of adapter:insert molar ratios and stock concentrations please contact Technical Support at sequencing.roche.com/support.

Dilution of KAPA Unique Dual-Indexed Adapters

- 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.
- Use the KAPA 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 Unique Dual-Indexed Adapters if needed. Adapters diluted in any other buffer or in PCR-grade water may not support optimal library construction efficiency.
- The KAPA Unique Dual-Indexed Adapter plate contains an excess of each adapter, over and above the stated volume of 20 µL. For this reason, and because diluted adapters are less stable, adapter dilutions must not be performed in the plate in which the adapters are supplied.
- Dilute only the amount of each adapter needed for same-day usage, in a new plate or tube. Long-term storage and multiple cycles of freezing and thawing of diluted adapter stocks are not recommended.
- The sealing foil provided with the KAPA Unique Dual-Indexed Adapter plate may be used for plates containing diluted adapters. Alternately, similar sealing foils may be used.
- For each batch of libraries to be constructed, prepare an appropriate volume of diluted adapter. Standard protocols call for 5 µL of appropriately diluted adapter stock per library.
  - If an adapter stock concentration >15 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.
  - An excess volume of each diluted adapter stock will be required to ensure accurate dispensing. The excess may be larger for automated vs. manual use.

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 instruments that employ patterned flow cells.
- Please follow the post-ligation cleanup instructions provided in the Operator Manual for your KAPA library preparation kit. While a single post-ligation cleanup with KAPA cleanup beads or Agencourt® AMPure® XP beads removes most unligated adapter and adapter-dimer (as recommended in KAPA HyperPrep, KAPA HyperPlus, KAPA EvoPrep and KAPA EvoPlus V2 Operator Manuals), a second cleanup or size selection step may be necessary to eliminate any remaining adapter species from the library. This may be particularly important for PCR-free workflows which do not offer the opportunity to remove unused adapter or adapter-dimer in the post-amplification cleanup. 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 the 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.

KAPA Unique Dual-Indexed Adapter Plate Layout

Figure 1 depicts the naming and placement of the 96 adapters in the 96-well plate. KAPA Unique Dual-Indexed Adapters are plated consecutively from well A to H in each column; and from column 1 to column 12. For example, well A1 contains UDI 01, well H1 contains UDI 08, well A2 contains UDI 09 and well H12 contains UDI 96.

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
A	UDI 01	UDI 09	UDI 17	UDI 25	UDI 33	UDI 41	UDI 49	UDI 57	UDI 65	UDI 73	UDI 81	UDI 89
B	UDI 02	UDI 10	UDI 18	UDI 26	UDI 34	UDI 42	UDI 50	UDI 58	UDI 66	UDI 74	UDI 82	UDI 90
C	UDI 03	UDI 11	UDI 19	UDI 27	UDI 35	UDI 43	UDI 51	UDI 59	UDI 67	UDI 75	UDI 83	UDI 91
D	UDI 04	UDI 12	UDI 20	UDI 28	UDI 36	UDI 44	UDI 52	UDI 60	UDI 68	UDI 76	UDI 84	UDI 92
E	UDI 05	UDI 13	UDI 21	UDI 29	UDI 37	UDI 45	UDI 53	UDI 61	UDI 69	UDI 77	UDI 85	UDI 93
F	UDI 06	UDI 14	UDI 22	UDI 30	UDI 38	UDI 46	UDI 54	UDI 62	UDI 70	UDI 78	UDI 86	UDI 94
G	UDI 07	UDI 15	UDI 23	UDI 31	UDI 39	UDI 47	UDI 55	UDI 63	UDI 71	UDI 79	UDI 87	UDI 95
H	UDI 08	UDI 16	UDI 24	UDI 32	UDI 40	UDI 48	UDI 56	UDI 64	UDI 72	UDI 80	UDI 88	UDI 96

Figure 1. Layout of the KAPA Unique Dual-Indexed Adapter plate. Detailed multiplexing guidelines are provided in Table 1a and 1b and sequencing indexes (barcodes) included in KAPA Unique Dual-Indexed Adapters are provided in Table 2.

KAPA Unique Dual-Indexed Adapter Index Sequences

Sequencing indexes (barcodes) included in KAPA Unique Dual-Indexed Adapters are given in Table 2. For convenience, all 96 index sequences in a comma-separated values file, as well as instructions for installation of KAPA Unique Dual-Indexed Adapter indexes for use with Illumina Experiment Manager are available. Please contact your local customer support center for a copy or submit a support request at sequencing.roche.com/support.

Pooling Guidelines

For low-plexity pooling applications (up to 8-plex) on Illumina sequencing platforms, specific index combinations must be used. As a rule, include two libraries in a low-plex pool that are indexed with two unique, fully color-balanced indices. This will prevent registration failure and laser color complexity issues during sequencing and de-multiplexing. To ensure equal sequencing read distributions in multiplexed applications, libraries must be carefully quantified and/or normalized prior to pooling for capture or cluster generation.

Guidelines for Illumina iSeq, MiniSeq, MiSeq, NextSeq 1000/2000 (standard SBS chemistry) & 500/550, NovaSeq 6000, HiSeq 2000/2500 & 3000/4000 and HiSeq X instruments:

Table 1a details the recommended multiplexing combinations.

- **Pooling two samples (two-plex):**
  - Two-plex sequencing using KAPA Unique Dual-Indexed Adapters is only recommended on two-channel Illumina instruments i.e. the MiniSeq, NextSeq, and NovaSeq instruments. **IMPORTANT! The recommended index combinations are different depending on the sequencer and/or reagent chemistry that is being used (Table 1a).**
  - Use only the recommended combinations listed in Table 1a. These are color-balanced.
  - *The following combinations are **not** color-balanced and are not recommended for two-plex applications on a MiniSeq (with Rapid Reagent kits), NextSeq 1000/2000 (standard SBS chemistry) or a NovaSeq 6000 instrument (with v1.0 reagent kits):*

» UDI 03 + 04 » UDI 31 + 32 » UDI 59 + 60 » UDI 93 + 94
» UDI 07 + 08 » UDI 37 + 38 » UDI 77 + 78 » UDI 95 + 96
» UDI 11 + 12 » UDI 43 + 44 » UDI 79 + 80
» UDI 21 + 22 » UDI 45 + 46 » UDI 81 + 82
» UDI 27 + 28 » UDI 53 + 54 » UDI 83 + 84

  - *The following combinations are **not** color-balanced and are not recommended for two-plex applications on a MiniSeq (with Standard reagent Kits), NextSeq 500/550 or a NovaSeq 6000 instrument (with v1.5 reagent kits):*

» UDI 01 + 02 » UDI 27 + 28 » UDI 51 + 52 » UDI 69 + 70
» UDI 03 + 04 » UDI 29 + 30 » UDI 53 + 54 » UDI 75 + 76
» UDI 07 + 08 » UDI 31 + 32 » UDI 57 + 58 » UDI 81 + 82

» UDI 09 + 10 » UDI 37 + 38 » UDI 59 + 60 » UDI 83 + 84
» UDI 11 + 12 » UDI 39 + 40 » UDI 61 + 62 » UDI 91 + 92
» UDI 21 + 22 » UDI 43 + 44 » UDI 63 + 64 » UDI 95 + 96
» UDI 23 + 24 » UDI 45 + 46 » UDI 65 + 66
» UDI 25 + 26 » UDI 49 + 50 » UDI 67 + 68

- **Pooling three samples (three-plex):**
  - Like with two-plex sequencing, three-plex sequencing using KAPA Unique Dual-Indexed Adapters is only recommended on two-channel Illumina instruments i.e. the MiniSeq, NextSeq, and NovaSeq instruments.
  - To obtain a three-plex combination, any recommended two-plex combination listed in Table 1a may be used with any other KAPA Unique Dual-Indexed Adapter on the plate. If the two-plex combination is color-balanced, the three-plex combination will also be color-balanced.
- **Pooling four samples (four-plex):**
  - Use only the recommended combinations listed in Table 1a (e.g., UDI 05 to UDI 08 or UDI 09 to UDI 12). These are color-balanced. **The following combinations are not color-balanced and are not recommended for four-plexes:** UDI 01 to UDI 04; UDI 21 to UDI 24; UDI 37 to UDI 40; UDI 61 to UDI 64; UDI 69 to UDI 72; and UDI 85 to UDI 88.
- **Pooling five samples (five-plex):**
  - Use only the recommended combinations listed in Table 1a (e.g., UDI 17 to UDI 21). These are color-balanced. **The following combinations are not color-balanced and are not recommended for five-plexes:** UDI 01 to UDI 05; UDI 20 to UDI 24; UDI 36 to UDI 40; UDI 68 to UDI 72; UDI 84 to UDI 88
- **Pooling six samples (six-plex):**
  - Use only the recommended combinations listed in Table 1a (e.g., UDI 17 to UDI 22). These are color-balanced. **The following combinations are not color-balanced and are not recommended for six-plexes:** UDI 01 to UDI 06; UDI 35 to UDI 40; UDI 67 to UDI 72.
- **Pooling seven samples (seven-plex):**
  - Use only the recommended combinations listed in Table 1a (e.g., UDI 17 to UDI 23). These are color-balanced. **The following combinations are not color-balanced and are not recommended for seven-plexes:** UDI 01 to UDI 07; UDI 34 to UDI 40; UDI 66 to UDI 72
- **Pooling eight samples (eight-plex):**
  - Use the eight KAPA Unique Dual-Indexed Adapters plated in any column (Figure 1; Table 1a).
- **Pooling larger than eight samples (up to 96-plex):**
  - Any eight-plex combination may be used with any other KAPA Unique Dual-Indexed Adapter.

Table 1a. Detailed pooling guidelines for Illumina iSeq, MiniSeq, MiSeq, NextSeq 1000/2000 (standard SBS chemistry) & NextSeq 500/550, NovaSeq 6000, HiSeq 2000/2500 & 3000/4000 and HiSeq X instruments

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
NOTE! Recommended two-plex combinations for sequencing on Illumina MiniSeq (with Rapid Reagent kits), NextSeq 1000/2000 (standard SBS chemistry) and NovaSeq 6000 (with v1.0 reagent kits)[1]											
01 + 02 or 05 + 06	09 + 10 or 13 + 14 or 15 + 16	17 + 18 or 19 + 20 or 23 + 24	25 + 26 or 29 + 30	33 + 34 or 35 + 36 or 39 + 40	41 + 42 or 47 + 48	49 + 50 or 51 + 52 or 55 + 56	57 + 58 or 61 + 62 or 63 + 64	65 + 66 or 67 + 68 or 69 + 70 or 71 + 72	73 + 74 or 75 + 76	85 + 86 or 87 + 88	89 + 90 or 91 + 92
NOTE! Recommended two-plex combinations for sequencing on Illumina MiniSeq (with Standard reagent kits), NextSeq 500/550 and NovaSeq 6000 (with v1.5 reagent kits)[1,2]											
05 + 06	13 + 14 or 15 + 16	17 + 18 or 19 + 20	None	33 + 34 or 35 + 36	41 + 42 or 47 + 48	55 + 56	None	71 + 72	73 + 74 or 77 + 78 or 79 + 80	85 + 86 or 87 + 88	89 + 90 or 93 + 94
Recommended three-plex combinations on Illumina MiniSeq, NextSeq 500/550 and 1000/2000[3] and NovaSeq 6000 (all reagent kits) instruments[1,2]											
Any recommended two-plex combination may be used with any other KAPA Unique Dual-Indexed Adapter											
Recommended four-plex combinations on all Illumina instruments[3]											
05 - 08	09 - 12 or 13 - 16	17 - 20	25 - 28 or 29 - 32	33 - 36	41 - 44 or 45 - 48	49 - 52 or 53 - 56	57 - 60	65 - 68	73 - 76 or 77 - 80	81 - 84	89 - 92 or 93 - 96
Recommended five-plex combinations on all Illumina instruments[3]											
04 - 08	09 - 13 or 12 - 16	17 - 21	25 - 29 or 28 - 32	33 - 37	41 - 45 or 44 - 48	49 - 53 or 52 - 56	57 - 61 or 60 - 64	65 - 69	73 - 77 or 76 - 80	81 - 85	89 - 93 or 92 - 96
Recommended six-plex combinations on all Illumina instruments[3]											
	09 - 14 or 11 - 16	17 - 22 or 19 - 24	25 - 30 or 27 - 32	33 - 38	41 - 46 or 43 - 48	49 - 54 or 51 - 56	57 - 62 or 59 - 64	65 - 70	73 - 78 or 75 - 80	81 - 86 or 83 - 88	89 - 94 or 91 - 96
Recommended seven-plex combinations on all Illumina instruments[3]											
	09 - 15 or 10 - 16	17 - 23 or 18 - 24	25 - 31 or 26 - 32	33 - 39	41 - 47 or 42 - 48	49 - 55 or 50 - 56	57 - 63 or 58 - 64	65 - 71	73 - 79 or 74 - 80	81 - 87 or 82 - 88	89 - 95 or 90 - 96
Recommended eight-plex combinations on all Illumina instruments[3]											
01 - 08	09 - 16	17 - 24	25 - 32	33 - 40	41 - 48	49 - 56	57 - 64	65 - 72	73 - 80	81 - 88	89 - 96
Recommended >eight-plex (up to 96-plex) combinations on all Illumina instrument[3]											
Any 8-plex combination may be used with any other KAPA Unique Dual-Indexed Adapter											

[1] Two- and three-plex sequencing using KAPA Unique Dual-Indexed Adapters is only recommended on two-channel Illumina instruments e.g., the MiniSeq, NextSeq, and NovaSeq instruments

[2] Illumina MiniSeq with Standard reagent kits, NextSeq 500/550, NextSeq 1000/2000 and NovaSeq 6000 (v1.5 reagent kits) instruments require the reverse complement orientation of the P5 index resulting in fewer recommended low-plex combinations

[3] Excludes Illumina NextSeq 1000/2000, NovaSeq X and X Plus instruments that make use of XLEAP-SBS chemistry

Guidelines for Illumina NextSeq 1000/2000, NovaSeq X and X Plus instruments that make use of XLEAP-SBS chemistry:

The NextSeq 1000/2000, NovaSeq X and X Plus uses blue/green chemistry. For low-plex applications (≤ 4-plex), ensure green signal (i.e. C or T index base) is present for each cycle of index sequencing to ensure appropriate color-balancing is maintained. Table 1b details the recommended three-plex* and four-plex combinations. To obtain a five-plex and higher combination, any recommended four-plex combination listed in Table 1b may be used with any other KAPA Unique Dual-Indexed Adapter on the plate. If the four-plex combination is color-balanced, the five-plex combination will also be color-balanced.

Table 1b. Detailed pooling guidelines for Illumina NextSeq 1000/2000, NovaSeq X and X Plus (XLEAP-SBS chemistry)

Recommended three, and four-plex combinations on Illumina NextSeq 1000/2000, NovaSeq X and X Plus	
3-plex	4-plex
UDI 06 + UDI 07 + UDI 19	UDI 01 + UDI 02 + UDI 03 + UDI 35
UDI 13 + UDI 14 + UDI 43	UDI 06 + UDI 07 + UDI 08 + UDI 19
UDI 15 + UDI 16 + UDI 33	UDI 10 + UDI 11 + UDI 12 + UDI 24
UDI 17 + UDI 18 + UDI 24	UDI 13 + UDI 14 + UDI 15 + UDI 32
UDI 27 + UDI 28 + UDI 49	UDI 16 + UDI 17 + UDI 18 + UDI 25
UDI 34 + UDI 35 + UDI 41	UDI 26 + UDI 27 + UDI 28 + UDI 33
UDI 37 + UDI 38 + UDI 48	UDI 36 + UDI 37 + UDI 38 + UDI 58
UDI 46 + UDI 47 + UDI 79	UDI 42 + UDI 43 + UDI 44 + UDI 64
UDI 50 + UDI 51 + UDI 72	UDI 45 + UDI 46 + UDI 47 + UDI 63
UDI 57 + UDI 58 + UDI 59	UDI 48 + UDI 49 + UDI 50 + UDI 72
UDI 61 + UDI 62 + UDI 90	UDI 51 + UDI 52 + UDI 53 + UDI 62
UDI 65 + UDI 66 + UDI 70	UDI 54 + UDI 55 + UDI 56 + UDI 59
UDI 68 + UDI 69 + UDI 82	UDI 65 + UDI 66 + UDI 67 + UDI 70
UDI 73 + UDI 74 + UDI 85	UDI 73 + UDI 74 + UDI 75 + UDI 85
UDI 80 + UDI 81 + UDI 88	UDI 77 + UDI 78 + UDI 79 + UDI 88
UDI 84 + UDI 86 + UDI 93	UDI 80 + UDI 81 + UDI 82 + UDI 92
UDI 91 + UDI 94 + UDI 96	UDI 83 + UDI 84 + UDI 86 + UDI 93
	UDI 89 + UDI 90 + UDI 94 + UDI 95

*There are no color-balanced two-plex combinations available. Consider using an alternative such as KAPA Universal Adapter and KAPA UDI Primer Mixes if the application allows.

Table 2. KAPA Unique Dual-Indexed Adapter index sequences (UDI 01 – UDI 32)[1]

Well position	Unique Dual-Indexed Adapter	P7 Index sequence (all Illumina instruments)	P5 Index sequence[2] [HiSeq 2000/2500, MiniSeq with Rapid Reagent kits, MiSeq, and NovaSeq 6000 (with v1.0 reagent kits) instruments]	P5 Index sequence[3] [iSeq, MiniSeq with Standard reagent kits, NextSeq systems, HiSeq 3000/4000, HiSeq X, NovaSeq X/Plus and NovaSeq 6000 (with v1.5 reagent kits) instruments]
A1	UDI 01	GTAACATC	CAGCGATT	AATCGCTG
B1	UDI 02	AGGTAAGG	CACGATTC	GAATCGTG
C1	UDI 03	ACAGGTAT	GCCACCAT	ATGGTGGC
D1	UDI 04	AATGTTCT	AGTCACCT	AGGTGACT
E1	UDI 05	TCTGCAAG	TTCACCTT	AAGGTGAA
F1	UDI 06	CAGCGGTA	TGACTTGG	CCAAGTCA
G1	UDI 07	CGCCTTCC	GCGGACTT	AAGTCCGC
H1	UDI 08	CAATAGTC	CAGCTCAC	GTGAGCTG
A2	UDI 09	ATTATCAA	CGACTCTC	GAGAGTCG
B2	UDI 10	CCAACATT	GCTCTCTT	AAGAGAGC
C2	UDI 11	GCCTAGCC	TTGGTCTG	CAGACCAA
D2	UDI 12	GACCAGGA	CTGGCTAT	ATAGCCAG
E2	UDI 13	CTGTAATC	AATTGCTT	AAGCAATT
F2	UDI 14	ACTAAGAC	TTCCAGCT	AGCTGGAA
G2	UDI 15	TCGCTAGA	AGTACTGC	GCAGTACT
H2	UDI 16	AACGCATT	GCAGGTTG	CAACCTGC
A3	UDI 17	TGCTGCTG	GTCCTCAT	ATGAGGAC
B3	UDI 18	TATCTGCC	CCAACGCT	AGCGTTGG
C3	UDI 19	ATTCCTCT	GCGATATT	AATATCGC
D3	UDI 20	CAACTCTC	ATCTTCTC	GAGAAGAT
E3	UDI 21	GCCGTCGA	TTAATCAC	GTGATTAA
F3	UDI 22	TATCCAGG	TCCACTTC	GAAGTGGA
G3	UDI 23	TAAGCACA	GACATTAA	TTAATGTC
H3	UDI 24	GTCCACAG	CGCGAATA	TATTCGCG
A4	UDI 25	ACACGATC	AATACCAT	ATGGTATT
B4	UDI 26	GTATAACA	TGCTTCAC	GTGAAGCA
C4	UDI 27	TGTCGGAT	TCAGGCTT	AAGCCTGA
D4	UDI 28	AGGATCTA	GAACTTCG	CGAAGTTC
E4	UDI 29	AGCAATTC	CTGCTCCT	AGGAGCAG
F4	UDI 30	CCTATGCC	CAAGCTTA	TAAGCTTG
G4	UDI 31	AAGGATGT	CACTTCAT	ATGAAGTG
H4	UDI 32	TTGAGCCT	TCATTCGA	TCGAATGA

[1] Sequencing indexes (barcodes) included in KAPA Unique Dual-Indexed Adapters are given in Table 2. For convenience, all 96 index sequences in a comma-separated values file (delimited text file), as well as instructions for installation of KAPA Unique Dual-Indexed Adapter indexes for use with Illumina Experiment Manager are available. Please contact your local customer support center for a copy or submit a support request at sequencing.roche.com/support.
[2] The sequence of the P5 index in the forward orientation required when completing the sample sheet for HiSeq 2000/2500, MiniSeq with Rapid Reagent kits, MiSeq, and NovaSeq 6000 (if using v1.0 reagent kits) instruments.
[3] The reverse complement sequence of the P5 index in the orientation required when completing the sample sheet for Illumina iSeq, MiniSeq with Standard reagent kits, NextSeq systems, HiSeq 3000/4000, HiSeq X, NovaSeq X/Plus and NovaSeq 6000 (v1.5 reagent kits) instruments.

Table 2. KAPA Unique Dual-Indexed Adapter index sequences (UDI 33 – UDI 64)[1]

Well position	Unique Dual-Indexed Adapter	P7 Index sequence (all Illumina instruments)	P5 Index sequence[2] [HiSeq 2000/2500, MiniSeq with Rapid Reagent kits MiSeq, and NovaSeq 6000 (v1.0 reagent kits) instruments]	P5 Index sequence[3] [iSeq, MiniSeq with Standard reagent kits, NextSeq systems, HiSeq 3000/4000, HiSeq X, NovaSeq X/Plus and NovaSeq 6000 (with v1.5 reagent kits) instruments]
A5	UDI 33	CACATCCT	GCTGCACT	AGTGCAGC
B5	UDI 34	TTCGCTGA	CGCATATT	AATATGCG
C5	UDI 35	CATGCTTA	ATGAATTA	TAATTCAT
D5	UDI 36	AAGTAGAG	ATCGACTG	CAGTCGAT
E5	UDI 37	CATAGCGA	GACGGTTA	TAACCGTC
F5	UDI 38	AGTTGCTT	TAGCATTG	CAATGCTA
G5	UDI 39	GCACATCT	AACCTCTT	AAGAGGTT
H5	UDI 40	CCTACCAT	GCTTCCTA	TAGGAAGC
A6	UDI 41	TGCTCGAC	ATCCTTAA	TTAAGGAT
B6	UDI 42	CCAGTTAG	CCTGTCAT	ATGACAGG
C6	UDI 43	TGTTCCGA	TTAGCCAG	CTGGCTAA
D6	UDI 44	GGTCCAGA	CGGTTCTT	AAGAACCG
E6	UDI 45	TCGGAATG	CTACATTG	CAATGTAG
F6	UDI 46	ATAGCGTC	TACTCCAG	CTGGAGTA
G6	UDI 47	AACTTGAC	GCTAGCAG	CTGCTAGC
H6	UDI 48	ATTCTAGG	TTCTTGGC	GCCAAGAA
A7	UDI 49	TTGAATAG	TCCATAAC	GTTATGGA
B7	UDI 50	TCTGGCGA	AATTCAAC	GTTGAATT
C7	UDI 51	TAATGAAC	CTTGGCTT	AAGCCAAG
D7	UDI 52	ATTATGTT	CTGTATTC	GAATACAG
E7	UDI 53	ATTGTCTG	TTCACAGA	TCTGTGAA
F7	UDI 54	GAAGAAGT	CTATTAGC	GCTAATAG
G7	UDI 55	GACAGTAA	GCGATTAC	GTAATCGC
H7	UDI 56	CCTTCGCA	CATCACTT	AAGTGATG
A8	UDI 57	CATGATCG	TACTCTCC	GGAGAGTA
B8	UDI 58	TCCTTGGT	GAATCGAC	GTCGATTC
C8	UDI 59	GTCATCTA	TCCAACCA	TGGTTGGA
D8	UDI 60	GAACCTAG	CTGGTATT	AATACCAG
E8	UDI 61	CAGCAAGG	CCTCTAAC	GTTAGAGG
F8	UDI 62	CGTTACCA	GAACGCTA	TAGCGTTC
G8	UDI 63	TCCAGCAA	AATTGGCC	GGCCAATT
H8	UDI 64	CAGGAGCC	GTCCAATC	GATTGGAC

[1] Sequencing indexes (barcodes) included in KAPA Unique Dual-Indexed Adapters are given in Table 2. For convenience, all 96 index sequences in a comma-separated values file (delimited text file), as well as instructions for installation of KAPA Unique Dual-Indexed Adapter indexes for use with Illumina Experiment Manager are available. Please contact your local customer support center for a copy or submit a support request at sequencing.roche.com/support.
[2] The sequence of the P5 index in the forward orientation required when completing the sample sheet for HiSeq 2000/2500, MiniSeq with Rapid Reagent kits, MiSeq, and NovaSeq 6000 (if using v1.0 reagent kits) instruments.
[3] The reverse complement sequence of the P5 index in the orientation required when completing the sample sheet for Illumina iSeq, MiniSeq with Standard reagent kits, NextSeq systems, HiSeq 3000/4000, HiSeq X, NovaSeq X/Plus and NovaSeq 6000 (v1.5 reagent kits) instruments.

Table 2. KAPA Unique Dual-Indexed Adapter index sequences (UDI 65 – UDI 96)[1]

Well position	Unique Dual-Indexed Adapter	P7 Index sequence (all Illumina instruments)	P5 Index sequence[2] [HiSeq 2000/2500, MiniSeq with Rapid Reagent kits, MiSeq, and NovaSeq 6000 (with v1.0 reagent kits) instruments]	P5 Index sequence[3] [iSeq, MiniSeq with Standard reagent kits, NextSeq systems, HiSeq 3000/4000, HiSeq X, NovaSeq X/Plus and NovaSeq 6000 (with v1.5 reagent kits) instruments]
A9	UDI 65	TTACGCAC	GACCATCT	AGATGGTC
B9	UDI 66	AGGTTATC	ATCATACC	GGTATGAT
C9	UDI 67	TCGCCTTG	GCTGATTC	GAATCAGC
D9	UDI 68	CCAGAGCT	CGAACTTC	GAAGTTCG
E9	UDI 69	TACTTAGC	AGGTACCA	TGGTACCT
F9	UDI 70	GTCTGATG	ATATCCGA	TCGGATAT
G9	UDI 71	TCTCGGTC	CTGACATC	GATGTCAG
H9	UDI 72	AAGACACT	TGACAGCA	TGCTGTCA
A10	UDI 73	CTACCAGG	CAACTGAT	ATCAGTTG
B10	UDI 74	ACTGTATC	TGCTATTA	TAATAGCA
C10	UDI 75	CTGTGGCG	CACTAGCC	GGCTAGTG
D10	UDI 76	TGTAATCA	AATCTCCA	TGGAGATT
E10	UDI 77	TTATATCT	GTCTGCAC	GTGCAGAC
F10	UDI 78	GCCGCAAC	TCATGTCT	AGACATGA
G10	UDI 79	TGTAACTC	CGACAGTT	AACTGTCG
H10	UDI 80	CTGCGGAT	GGTTATCT	AGATAACC
A11	UDI 81	GACCGTTG	CCATCACA	TGTGATGG
B11	UDI 82	AACAATGG	TAGTTAGC	GCTAACTA
C11	UDI 83	AGGTGCGA	CTTCTGGC	GCCAGAAG
D11	UDI 84	AGGTCGCA	GCACAATT	AATTGTGC
E11	UDI 85	ACCAACTG	GGCAATAC	GTATTGCC
F11	UDI 86	TGCAAGTA	CCAACTAA	TTAGTTGG
G11	UDI 87	GACCTAAC	GCTCACCA	TGGTGAGC
H11	UDI 88	AGCATGGA	AGCGCTAA	TTAGCGCT
A12	UDI 89	ACAGTTGA	GCTCCGAT	ATCGGAGC
B12	UDI 90	TTGTCTAT	CTTGAATC	GATTCAAG
C12	UDI 91	CGCTATGT	TCCGCATA	TATGCGGA
D12	UDI 92	TTAATCAG	CCAATCTG	CAGATTGG
E12	UDI 93	CTATGCGT	GAATATCA	TGATATTC
F12	UDI 94	GATATCCA	GGATTAAC	GTTAATCC
G12	UDI 95	GAAGGAAG	CATCCTGG	CCAGGATG
H12	UDI 96	CTAACTCG	TATGGTTC	GAACCATA

[1] Sequencing indexes (barcodes) included in KAPA Unique Dual-Indexed Adapters are given in Table 2. For convenience, all 96 index sequences in a comma-separated values file (delimited text file), as well as instructions for installation of KAPA Unique Dual-Indexed Adapter indexes for use with Illumina Experiment Manager are available. Please contact your local customer support center for a copy or submit a support request at sequencing.roche.com/support.
[2] The sequence of the P5 index in the forward orientation required when completing the sample sheet for HiSeq 2000/2500, MiniSeq with Rapid Reagent kits, MiSeq, and NovaSeq 6000 (if using v1.0 reagent kits) instruments.
[3] The reverse complement sequence of the P5 index in the orientation required when completing the sample sheet for Illumina iSeq, MiniSeq with Standard reagent kits, NextSeq systems, HiSeq 3000/4000, HiSeq X, NovaSeq X/Plus and NovaSeq 6000 (v1.5 reagent kits) instruments.

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