

KAPA Unique Dual-Indexed Adapter Kit

Illumina® Platforms

KR1736 – v4.22

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

| | |
|--|----|
| Product Description | 2 |
| Product Applications | 2 |
| Product Specifications | 2 |
| Shipping and Storage | 2 |
| Quality Control | 2 |
| Important Parameters | 3 |
| Best Practices | 3 |
| Procedure for Handling KAPA Unique Dual-Indexed Adapter Plates | 3 |
| Additional Information pertaining to the use of KAPA Unique Dual-Indexed Adapter Plates on Automated platforms | 3 |
| Compatibility with KAPA Library Preparation Kits | 4 |
| KAPA Unique Dual-Indexed Adapter Working Concentrations | 4 |
| Adapter Concentration Calculations | 4 |
| Dilution of KAPA Unique Dual-Indexed Adapters | 5 |
| Post-ligation Processing | 5 |
| KAPA Unique Dual-Indexed Adapter Plate Layout | 6 |
| Pooling Guidelines | 6 |
| KAPA Unique Dual-Indexed Adapter Index Sequences | 8 |
| Restrictions and Liabilities | 11 |
| Note to Purchaser: Limited Product Warranty | 11 |
| Note to Purchaser: Limited License | 11 |

| 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 coverage is included for use on automated liquid handlers

| Quick Notes |
|--|
| <ul style="list-style-type: none"> • 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.

¹Illumina. 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 on dialog.roche.com, or upon request from the local Roche office.

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 coverage 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 (pierceable 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 Kits
- KAPA HTP and LTP Library Preparation 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 Technical Data Sheet 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:insert molar ratios in the range of 10:1 – 40:1 are recommended for KAPA HTP and LTP Library Preparation Kits, whereas KAPA HyperPrep, KAPA HyperPlus, KAPA EvoPlus Kits and KAPA RNA HyperPrep Kits are compatible with much higher ratios ($\geq 100:1$).

Very high adapter:insert molar ratios (200:1 – 1000:1) may be beneficial for low-input library construction with KAPA HyperPrep, KAPA HyperPlus and KAPA EvoPlus Kits.

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 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 2.4 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 μ L) 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 μ L. 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 μ L of appropriately diluted adapter stock per library.
 - If an adapter stock concentration $>15 \mu$ M 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 μ L 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 Technical Data Sheet 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 and KAPA EvoPlus Technical Data Sheets), 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. Detailed multiplexing guidelines are provided in Table 1 and sequencing indexes (barcodes) included in KAPA Unique Dual-Indexed Adapters are provided in Table 2.

| | 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 1 and sequencing indexes (barcodes) included in KAPA Unique Dual-Indexed Adapters are provided in Table 2.

Pooling Guidelines

For low-plexity pooling applications (up to 8-plex) on Illumina sequencing platforms, specific index combinations must be used. Table 1 details the recommended multiplexing combinations. 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.

• 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 1).

- » 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

- Use only the recommended combinations listed in Table 1. 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 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

• 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 1 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 1 (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 1 (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 1 (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 1 (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 1).
- **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 1. Detailed pooling guidelines

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

KAPA Unique Dual-Indexed Adapter Kit

Illumina® Platforms

Technical Data Sheet

KAPA Unique Dual-Indexed Adapter Index Sequences

Sequencing indexes (barcodes) included in KAPA Unique Dual-Indexed Adapters are given in Table 2 (p. 8 to p. 10). 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.

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

| Well position | Unique Dual-Indexed Adapter | P7 Index sequence (all Illumina instruments) | P5 Index sequence ¹ [HiSeq 2000/2500, MiniSeq with Rapid Reagent kits, MiSeq, NextSeq 1000/2000 and NovaSeq 6000 (with v1.0 reagent kits) instruments] | P5 Index sequence ² [iSeq, MiniSeq with Standard reagent kits, NextSeq 500/550, HiSeq 3000/4000, HiSeq X 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 | GAAGTTCTG | 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 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, NextSeq 1000/2000 and NovaSeq 6000 (if using v1.0 reagent kits) instruments.

² 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 500/550, HiSeq 3000/4000, HiSeq X and NovaSeq 6000 (v1.5 reagent kits) instruments.

KAPA Unique Dual-Indexed Adapter Kit

Illumina® Platforms

Technical Data Sheet

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, NextSeq 1000/2000 and NovaSeq 6000 (v1.0 reagent kits) instruments] | P5 Index sequence ³ [iSeq, MiniSeq with Standard reagent kits, NextSeq 500/550, HiSeq 3000/4000, HiSeq X 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, NextSeq 1000/2000 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 500/550, HiSeq 3000/4000, HiSeq X and NovaSeq 6000 (v1.5 reagent kits) instruments.

KAPA Unique Dual-Indexed Adapter Kit

Illumina® Platforms

Technical Data Sheet

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, NextSeq 1000/2000 and NovaSeq 6000 (with v1.0 reagent kits) instruments] | P5 Index sequence ³ [iSeq, MiniSeq with Standard reagent kits, NextSeq 500/550, HiSeq 3000/4000, HiSeq X 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 | AACTGTCC |
| 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, NextSeq 1000/2000 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 500/550, HiSeq 3000/4000, HiSeq X and NovaSeq 6000 (v1.5 reagent kits) instruments.

Restrictions and Liabilities

This Technical Data Sheet is provided “as is” and Kapa Biosystems assumes no responsibility for any typographical, technical, or other inaccuracies. The document is subject to change, without notice, in future editions.

To the maximum extent permitted by applicable law, Kapa Biosystems disclaims all warranties, either express or implied, with regard to this technical data sheet and any information contained herein, including but not limited to the implied warranties of merchantability and fitness for a particular purpose. Kapa Biosystems shall not be liable for errors or for incidental or consequential damages in connection with the furnishing, use, or performance of this document or of any information contained herein.

This document might contain references to third party sources of information, hardware or software, products, or services and/or third party web sites (collectively the “Third-Party Information”). Kapa Biosystems does not control, and is not responsible for, any Third-Party Information. The inclusion of Third-Party Information in this document does not imply endorsement by Kapa Biosystems of the Third-Party Information or the third party in any way.

Kapa Biosystems is not responsible nor will be liable in any way for your use of any software or equipment that is not supplied by Kapa Biosystems in connection with your use of Kapa Biosystems products.

Kapa Biosystems does not in any way guarantee or represent that you will obtain satisfactory results from using Kapa Biosystems products as described herein. The only warranties provided to you are included in the Limited Warranty enclosed with this document. You assume all risk in connection with your use of Kapa Biosystems products.

Note to Purchaser: Limited Product Warranty

Any product that does not meet the performance standards stated in the product specification sheet will be replaced at no charge. This warranty limits our liability to the replacement of the product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Kapa Biosystems. Kapa Biosystems shall have no liability for any direct, indirect, consequential or incidental damages arising out of the use, the results of use or the inability to use any product.

Note to Purchaser: Limited License

KAPA Unique Dual-Indexed Adapter Kits are developed, designed and sold exclusively for research purposes. Neither the product, nor any individual component, has been tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to the SDS, which is available on request. Certain applications of this product are covered by patents issued to parties other than Kapa Biosystems and applicable in certain countries. Purchase of this product does not include a license to perform any such applications. Users of this product may therefore be required to obtain a patent license depending upon the particular application and country in which the product is used.



Manufacturing, R & D
Cape Town, South Africa
Tel: +27 21 448 8200
Fax: +27 21 448 6503

Technical Support
sequencing.roche.com/support

© 2022 Roche Sequencing Solutions, Inc. All Rights Reserved.

KAPA, KAPA EVOPLUS, KAPA HYPERPREP and KAPA HYPERPLUS are trademarks of Roche. All other product names and trademarks are the property of their respective owners