



KAPA Adapter Kits

Ion Torrent™ Platforms

KR0574 – v3.16

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KAPA/Roche Kit Codes and Components		
KK8330 07962002001 8 libraries	10 µM Adapter P1 10 µM Adapter A	80 µL 80 µL
KK8331 07962029001 48 libraries	10 µM Adapter P1 10 µM Adapter A Barcodes 1 – 8	480 µL 60 µL
KK8332 07962037001 48 libraries	10 µM Adapter P1 10 µM Adapter A Barcodes 9 – 16	480 µL 60 µL
KK8333 07962045001 48 libraries	10 µM Adapter P1 10 µM Adapter A Barcodes 17 – 24	480 µL 60 µL
KK8334 07962053001 48 libraries	10 µM Adapter P1 10 µM Adapter A Barcodes 25 – 32	480 µL 60 µL
KK8335 07962061001 48 libraries	10 µM Adapter P1 10 µM Adapter A Barcodes 33 – 40	480 µL 60 µL
KK8336 07962070001 48 libraries	10 µM Adapter P1 10 µM Adapter A Barcodes 41 – 48	480 µL 60 µL
KK8337 07962088001 48 libraries	10 µM Adapter P1 10 µM Adapter A Barcodes 49 – 56	480 µL 60 µL
KK8338 07962096001 48 libraries	10 µM Adapter P1 10 µM Adapter A Barcodes 57 – 64	480 µL 60 µL
KK8339 07962100001 48 libraries	10 µM Adapter P1 10 µM Adapter A Barcodes 65 – 72	480 µL 60 µL
KK8340 07962118001 48 libraries	10 µM Adapter P1 10 µM Adapter A Barcodes 73 – 80	480 µL 60 µL
KK8341 07962126001 48 libraries	10 µM Adapter P1 10 µM Adapter A Barcodes 81 – 88	480 µL 60 µL
KK8342 07962134001 48 libraries	10 µM Adapter P1 10 µM Adapter A Barcodes 89 – 96	480 µL 60 µL

Quick Notes
<ul style="list-style-type: none"> • Barcoded adapter kits contain a sufficient amount of each barcoded adapter to prepare 6 libraries, for a total of 48 libraries per kit. • Adapters are duplexed oligonucleotides, which should not be heated above room temperature in order to avoid strand dissociation. • Avoid cross-contamination of barcoded adapters by careful handling of tubes and adhering to good laboratory practices. • Prior to pooling barcoded libraries for multiplexed sequencing, it is essential to normalize the molar concentration of the libraries to ensure that an equal number of reads is generated for each library. qPCR-based library quantification with the KAPA Library Quantification Kit for Ion Torrent is recommended.

Product Description

KAPA Adapter Kits for Ion Torrent platforms are designed for use with KAPA Library Preparation Kits for Ion Torrent platforms. Kits contain Adapter P1 in combination with either the non-barcoded Adapter A (KK8330), or a set of eight barcoded A adapters (KK8331 – KK8342). Libraries prepared using these adapters are suitable for sequencing on the Ion Personal Genome Machine™ and Ion Proton™ semiconductor sequencers.

Barcoded adapters allow for pooling of multiple fragment libraries prior to emulsion PCR (emPCR) in order to conduct multiplexed sequencing on a single chip. This simplifies the next generation sequencing workflow for a wide range of applications. Multiplexed sequencing also reduces the costs associated with emPCR, enrichment, and sequencing.

The sequences of the DNA barcodes in the barcoded KAPA Adapter Kits are identical to the sequences of the equivalent Ion Xpress™ barcodes and are thus optimized for equal representation of all barcodes in a pool. De-multiplexing of sequencing data is performed automatically by the Ion Torrent software. The highly dissimilar sequences of the barcodes minimize the possibility of incorrectly calling the barcode of a read due to sequencing or base calling errors.

Product Applications

KAPA Adapter Kits for Ion Torrent platforms are intended for use with the KAPA Library Preparation Kits for Ion Torrent platforms to generate libraries for either standard or multiplexed sequencing. Applications include whole genome shotgun sequencing, targeted sequencing by solution hybrid selection, RNA-seq, CHIP-seq, and amplicon sequencing.

Product Specifications

Shipping and Storage

KAPA 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 should not be heated 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.

Handling

Always ensure that adapters have been fully thawed and thoroughly mixed before use.

Quality Control

KAPA Adapters for Ion Torrent platforms are confirmed to contain less than 0.05% cross-contaminating adapter species, by deep sequencing using the Ion PGM™. Due to the sensitive nature of massively parallel sequencing, even extremely low levels of cross-contaminating barcoded adapters are detectable. Barcode cross-contamination can potentially confound sample de-multiplexing. It may also lead to spurious results, especially when simultaneously sequencing samples which are only subtly different, as in rare variant detection experiments. For more information, please contact Technical Support at kapabiosystems.com/support.

Important Parameters

Adapter Concentrations

- The recommended adapter concentration is dependent on the amount of input DNA and the median fragment size of the library. As a general guideline, an adapter:insert molar ratio of between 10:1 and 20:1 is recommended.
- The recommended adapter concentrations for 130, 260, 320, and 410 bp inserts prepared from 100 ng – 1 µg of input DNA are provided in Table 1.
- KAPA Adapters are supplied at a concentration of 10 µM. When 10 µL of each adapter is used per 70 µL Ligation and Nick Repair reaction, the final concentration of each adapter is 1.4 µM.
- If a lower final concentration is required, a dilution of the 10 µM adapters to the appropriate concentration is recommended, such that addition of 10 µL of each diluted adapter to a 70 µL Ligation and Nick Repair reaction will result in the recommended final adapter concentration, as shown in Table 1.
- While it is not necessary to adjust adapter concentrations to accommodate moderate sample-to-sample variation in input DNA quantity, using an adapter concentration that is appropriate for the molar concentration of input DNA is recommended.
- It is important to maintain an adapter:insert ratio of ≥10:1 in order to minimize the formation of chimeric library inserts. Conversely, adapter:insert ratios higher than 20:1 may lead to reduced library yields.

Multiplexed Sequencing of Barcoded Libraries

Before pooling barcoded libraries for multiplexed sequencing, the concentration of each barcoded library should be determined accurately using qPCR (KAPA Library Quantification Kit for Ion Torrent), fluorometry or electrophoresis (e.g., Bioanalyzer).

After quantification, prepare an equimolar pool of barcoded libraries. Preparation of an equimolar pool can be achieved by first normalizing the individual libraries to the same concentration—before pooling equal volumes of each library.

Quantify the final library pool prior to template preparation using qPCR (KAPA Library Quantification Kit for Ion Torrent), fluorometry or electrophoresis (e.g., Bioanalyzer).

Adapter Sequences

Adapter P1

```
5'          CCACTACGCCTCCGCTTTCCTCTCTATGGGCAGTCGGTGAT 3'
3'  T*T*GGTGATGCGGAGGCGAAAGGAGAGATACCCGTCAGCCACTA 5'
```

Adapter A

```
5'          CCATCTCATCCCTGCGTGTCTCCGACTCAG 3'
3'  T*T*GGTAGAGTAGGGACGCACAGAGGCTGAGTC 5'
```

Adapter A Barcode 1

```
5'  CCATCTCATCCCT*G*CGTGTCTCCGACTCAGCTAAGGTAACGAT 3'
3'  C-GCACAGAGGCTGAGTCGATTCCATTGCTA 5'
```

* phosphorothioate bond
- sequence alignment gap

The underlined portion of Adapter A Barcode 1 represents the position of the barcode, the sequence of which will vary as shown in Table 2. Aside from the unique barcode sequence, barcoded A adapters are identical.

Sequences of libraries produced with the barcoded adapter begin with the sequence TCAG[Barcode]GAT, followed by the insert sequence. The sequences of barcodes 1 – 96 are provided in Table 2.

Table 1. Recommended adapter concentrations (10 µL of stock per 70 µL Ligation and Nick Repair reaction)

Insert DNA per reaction	Recommended adapter concentration for DNA with a median fragment size of							
	130 bp		260 bp		320 bp		410 bp	
	Stock	Final	Stock	Final	Stock	Final	Stock	Final
1 µg	10 µM	1.4 µM	10 µM	1.4 µM	5 µM	0.7 µM	5 µM	0.7 µM
500 ng	5 µM	0.7 µM	5 µM	0.7 µM	2.5 µM	0.36 µM	2.5 µM	0.36 µM
100 ng	1 µM	0.14 µM	1 µM	0.14 µM	0.5 µM	0.07 µM	0.5 µM	0.07 µM

Table 2. Barcode sequences

Barcoded Adapter (1 – 8) KK8331		Barcoded Adapter (9 – 16) KK8332		Barcoded Adapter (17 – 24) KK8333	
Barcode 1	CTAAGGTAAC	Barcode 9	TGAGCGGAAC	Barcode 17	TCTATTCGTC
Barcode 2	TAAGGAGAAC	Barcode 10	CTGACCGAAC	Barcode 18	AGGCAATTGC
Barcode 3	AAGAGGATTC	Barcode 11	TCCTCGAATC	Barcode 19	TTAGTCGGAC
Barcode 4	TACCAAGATC	Barcode 12	TAGGTGGTTC	Barcode 20	CAGATCCATC
Barcode 5	CAGAAGGAAC	Barcode 13	TCTAACGGAC	Barcode 21	TCGCAATTAC
Barcode 6	CTGCAAGTTC	Barcode 14	TTGGAGTGTC	Barcode 22	TTCGAGACGC
Barcode 7	TTCGTGATTC	Barcode 15	TCTAGAGGTC	Barcode 23	TGCCACGAAC
Barcode 8	TTCCGATAAC	Barcode 16	TCTGGATGAC	Barcode 24	AACCTCATTC
Barcoded Adapter (25 – 32) KK8334		Barcoded Adapter (33 – 40) KK8335		Barcoded Adapter (41 – 48) KK8336	
Barcode 25	CCTGAGATAAC	Barcode 33	TTCTCATTGAAC	Barcode 41	TTCCACTTCCG
Barcode 26	TTACAACCTC	Barcode 34	TCGCATCGTTC	Barcode 42	AGCACGAATC
Barcode 27	AACCATCCGC	Barcode 35	TAAGCCATTGTC	Barcode 43	CTTGACACCGC
Barcode 28	ATCCGGAATC	Barcode 36	AAGGAATCGTC	Barcode 44	TTGGAGGCCAGC
Barcode 29	TCGACCACTC	Barcode 37	CTTGAGAATGTC	Barcode 45	TGGAGCTTCTC
Barcode 30	CGAGGTTATC	Barcode 38	TGGAGGACGGAC	Barcode 46	TCAGTCCGAAC
Barcode 31	TCCAAGCTGC	Barcode 39	TAACAATCGGC	Barcode 47	TAAGGCAACCAC
Barcode 32	TCTTACACAC	Barcode 40	CTGACATAATC	Barcode 48	TTCTAAGAGAC
Barcoded Adapter (49 – 56) KK8337		Barcoded Adapter (57 – 64) KK8338		Barcoded Adapter (65 – 72) KK8339	
Barcode 49	TCCTAACATAAC	Barcode 57	TCTGGCAACGGC	Barcode 65	TCCTGGCACATC
Barcode 50	CGGACAATGGC	Barcode 58	TCCTAGAACAC	Barcode 66	CCGCAATCATC
Barcode 51	TTGAGCCTATTC	Barcode 59	TCCTTGATGTTT	Barcode 67	TTCTACCAGTC
Barcode 52	CCGCATGGAAC	Barcode 60	TCTAGCTCTTC	Barcode 68	TCAAGAAGTTC
Barcode 53	CTGGCAATCCTC	Barcode 61	TCACTCGGATC	Barcode 69	TTCAATTGGC
Barcode 54	CCGGAAGAATCGC	Barcode 62	TTCTGCTTCAC	Barcode 70	CCTACTGGTC
Barcode 55	TCCACCTCCTC	Barcode 63	CCTTAGAGTTC	Barcode 71	TGAGGCTCCGAC
Barcode 56	CAGCATTAATTC	Barcode 64	CTGAGTTCCGAC	Barcode 72	CGAAGGCCACAC
Barcoded Adapter (73 – 80) KK8340		Barcoded Adapter (81 – 88) KK8341		Barcoded Adapter (89 – 96) KK8342	
Barcode 73	TCTGCCTGTC	Barcode 81	CCTGCCATTCGC	Barcode 89	TCCTGAATCTC
Barcode 74	CGATCGGTTC	Barcode 82	TTGGCATCTC	Barcode 90	CTAACACCGGC
Barcode 75	TCAGGAATAC	Barcode 83	CTAGGACATTC	Barcode 91	CGGAAGGATGC
Barcode 76	CGGAAGAACCTC	Barcode 84	CTTCCATAAC	Barcode 92	CTAGGAACCGC
Barcode 77	CGAAGCGATTC	Barcode 85	CCAGCCTCAAC	Barcode 93	CTTGTCCAATC
Barcode 78	CAGCCAATTCTC	Barcode 86	CTTGGTTATTC	Barcode 94	TCCGACAAGC
Barcode 79	CCTGGTTGTC	Barcode 87	TTGGCTGGAC	Barcode 95	CGGACAGATC
Barcode 80	TCGAAGGCAGGC	Barcode 88	CCGAACACTTC	Barcode 96	TTAAGCGGTC

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