KAPA Adapter Kits
Ion Torrent™ Platforms

KR0574 – v4.17

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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 – KK8333). 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.

KAPA/Roche Kit Codes and Components

<table>
<thead>
<tr>
<th>KAPA/Roche Kit Codes</th>
<th>Component Details</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>KK8330 07962002001</td>
<td>8 libraries</td>
<td>10 µM Adapter P1 80 µL 10 µM Adapter A 80 µL</td>
</tr>
<tr>
<td>KK8331 07962029001</td>
<td>48 libraries</td>
<td>10 µM Adapter P1 480 µL 10 µM Adapter A Barcodes 1 – 8 60 µL</td>
</tr>
<tr>
<td>KK8332 07962037001</td>
<td>48 libraries</td>
<td>10 µM Adapter P1 480 µL 10 µM Adapter A Barcodes 9 – 16 60 µL</td>
</tr>
<tr>
<td>KK8333 07962045001</td>
<td>48 libraries</td>
<td>10 µM Adapter P1 480 µL 10 µM Adapter A Barcodes 17 – 24 60 µL</td>
</tr>
</tbody>
</table>

Quick Notes
- 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 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.
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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 -15ºC to -25ºC 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 sequencing.roche.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.

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

<table>
<thead>
<tr>
<th>Insert DNA per reaction</th>
<th>Stock Final</th>
<th>Stock Final</th>
<th>Stock Final</th>
<th>Stock Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>130 bp</td>
<td>10 µM 1.4 µM</td>
<td>10 µM 1.4 µM</td>
<td>5 µM 0.7 µM</td>
<td>5 µM 0.7 µM</td>
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<tr>
<td>260 bp</td>
<td>5 µM 0.7 µM</td>
<td>5 µM 0.7 µM</td>
<td>2.5 µM 0.36 µM</td>
<td>2.5 µM 0.36 µM</td>
</tr>
<tr>
<td>320 bp</td>
<td>0.5 µM 0.07 µM</td>
<td>0.5 µM 0.07 µM</td>
<td>0.5 µM 0.07 µM</td>
<td>0.5 µM 0.07 µM</td>
</tr>
<tr>
<td>410 bp</td>
<td>1 µM 0.14 µM</td>
<td>1 µM 0.14 µM</td>
<td>0.5 µM 0.07 µM</td>
<td>0.5 µM 0.07 µM</td>
</tr>
</tbody>
</table>
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' CCACCTACGCCTCCGCTTTCCCTCTATGGGCAGTCGGTGAT 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 TCA[GAT]GAT, followed by the insert sequence. The sequences of barcodes 1 – 24 are provided in Table 2.

Table 2. Barcode sequences

<table>
<thead>
<tr>
<th>Barcoded Adapter (1 – 8)</th>
<th>Barcode 1</th>
<th>Barcode 2</th>
<th>Barcode 3</th>
<th>Barcode 4</th>
<th>Barcode 5</th>
<th>Barcode 6</th>
<th>Barcode 7</th>
<th>Barcode 8</th>
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</thead>
<tbody>
<tr>
<td>KK8331</td>
<td>CTAAGGTAAC</td>
<td>TAAAGGAAC</td>
<td>AAGAGGATTC</td>
<td>TACCAAGATC</td>
<td>CAGAAGGAAC</td>
<td>CGTCAAGTTC</td>
<td>TCGTGATTTC</td>
<td>TCCCGATAAC</td>
</tr>
<tr>
<td>Barcoded Adapter (9 – 16)</td>
<td>Barcode 9</td>
<td>Barcode 10</td>
<td>Barcode 11</td>
<td>Barcode 12</td>
<td>Barcode 13</td>
<td>Barcode 14</td>
<td>Barcode 15</td>
<td>Barcode 16</td>
</tr>
<tr>
<td>KK8332</td>
<td>TGAGCGGAAC</td>
<td>CTGACCGAAC</td>
<td>TCCTCGAATC</td>
<td>TAGGTGGTTC</td>
<td>TCTACCGGAC</td>
<td>TGGAGTTGTC</td>
<td>TCAAGGATGTC</td>
<td>TCTGGATAAC</td>
</tr>
</tbody>
</table>
| KK8333                   | TCTATTCGTC| AGGCAATTGC| TAGTCGGAC  | CAGATCCATC| TCGCAATTAC| TCGAGAGGC| TGCACCGAAC| AACCTCATT
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