

Product Description

The KAPA HyperPlus Kit provides a versatile, streamlined DNA fragmentation and library construction protocol for the rapid preparation of libraries for Illumina sequencing. The novel one-tube chemistry and protocol improves the efficiency and consistency of library construction, and yields libraries of similar or better quality than those produced with the KAPA Hyper Prep Kit from Covaris-sheared DNA. It outperforms fragmentation-based workflows in terms of robustness, flexibility and sequence coverage and uniformity.

The workflow combines enzymatic steps and employs minimal bead-based cleanups, thereby reducing sample handling and overall library preparation time to 1.5 – 3 hrs. The kit contains all of the enzymes and reaction buffers required for:

1. enzymatic fragmentation to produce dsDNA fragments;
2. end repair and A-tailing to produce end-repaired, 5'-phosphorylated, 3'-dA-tailed dsDNA fragments;
3. adapter ligation, during which dsDNA adapters with 3'-dTTP overhangs are ligated to 3'-dA-tailed molecules; and
4. library amplification (optional), which employs high-fidelity, low-bias PCR to amplify library fragments carrying appropriate adapter sequences on both ends.

The kit provides a single concentrated buffer and a single enzyme mixture for enzymatic fragmentation, as well as for each of the two library construction steps—but does not include adapters or beads required for cleanups after adapter ligation and library amplification. KAPA Pure Beads and KAPA Adapters are sold separately.

In order to maximize sequence coverage uniformity, it is critical to minimize library amplification bias. KAPA HiFi DNA Polymerase is designed for low-bias, high-fidelity PCR, and is the reagent of choice for NGS library amplification.^{1,2,3,4} KAPA HyperPlus Kits include KAPA HiFi HotStart ReadyMix (2X), a ready-to-use PCR mix comprising all the components for library amplification—except primers and template. Kits also include Library Amplification Primer Mix (10X), designed for the high-efficiency amplification of Illumina libraries flanked by adapters containing the P5 and P7 flow cell sequences. Kits without the amplification module (KK8511, KK8513, KK8515) are available for PCR-free workflows. They may also be combined with KAPA HiFi Real-time Library Amplification Kits (KK2701, KK2702) for more precise control over library amplification.

1. Oyola, S.O., et al., *BMC Genomics* 13, 1 (2012).
2. Quail, M.A., et al., *Nature Methods* 9, 10 (2012).
3. Quail, M.A., et al., *BMC Genomics* 13, 341 (2012).
4. Ross, M.G., et al., *Genome Biology* 14, R51 (2013).

Product Applications

KAPA HyperPlus Kits are ideally suited for low- and high-throughput NGS library construction workflows that require DNA fragmentation, end repair, A-tailing, adapter ligation and library amplification (optional). Kits are designed for library construction from a wide range of sample types and inputs (1 ng – 1 µg), and are compatible with complex, genomic DNA; low-complexity samples such as small viral genomes, plasmids, cDNA and long amplicons; and low-quality DNA such as FFPE samples.

The entire workflow is automation-friendly and may be incorporated into workflows for a wide range of NGS applications, including:

- whole-genome, shotgun sequencing
- whole exome or targeted sequencing, using Roche SeqCap EZ, Agilent SureSelect, Illumina TruSeq, or IDT xGen Lockdown Probes, or other hybridization capture systems
- RNA-seq (starting with cDNA).

Product Specifications

Shipping and Storage

The enzymes provided in this kit are temperature sensitive, and appropriate care should be taken during shipping and storage. KAPA HyperPlus Kits are shipped on dry ice. Upon receipt, immediately store enzymes and reaction buffers at -15°C to -25°C in a constant-temperature freezer. When stored under these conditions and handled correctly, the kit components will retain full activity until the expiry date indicated on the kit label.

Handling

Always ensure that KAPA HyperPlus Kit components have been fully thawed and thoroughly mixed before use. The End Repair & A-Tailing Buffer and Ligation Buffer may contain precipitates when thawed at 2°C to 8°C. These buffers must be thawed at room temperature and vortexed thoroughly before use. KAPA HiFi HotStart ReadyMix (2X) contains isostabilizers and may not freeze completely, even when stored at -15°C to -25°C. Nevertheless, always ensure that the ReadyMix is fully thawed and thoroughly mixed before use. Reaction master mixes prepared from the enzymes and buffers for fragmentation, end repair and A-tailing, as well as for ligation, are very viscous and require special attention during pipetting. Keep all enzyme components and master mixes on ice as long as possible during handling and preparation.

Quality Control

All kit components are subjected to stringent functional quality control, are free of detectable contaminating exo- and endonuclease activities, and meet strict requirements with respect to DNA contamination. Please contact Technical Support at sequencing.roche.com/support for more information.

KAPA *HyperPlus* rinkinys užtikrina universalią pradinės DNR fragmentaciją bei pateikia NGS bibliotekos konstravimo protokolą ir suteikia galimybę greitai paruošti NGS bibliotekas, suderinamas su *Illumina* sekoskaitos platformomis. Inovatyvūs viename mėgintuvėlyje vykstantys cheminiai procesai ir pateikiamas protokolas padidina bibliotekų sudarymo efektyvumą, tokiu būdu gaunant panašios ar geresnės kokybės bibliotekas, nei tos, kurios ruošiamos naudojant KAPA *Hyper Prep* rinkinį su *Covaris* fragmentuota DNR.

Bibliotekų paruošimo procesą sudaro fermentiniai procesai bei minimalus valymas naudojant magnetines daleles, todėl mėginio paruošimo ir bendras bibliotekos paruošimo laikas sutrumpėja iki 1,5–3 val. Rinkinyje yra visi fermentai ir reakcijų buferiai, kurių reikia:

1. fermentinei fragmentacijai, kad būtų gauti dsDNR fragmentai;
2. DNR galų paruošimui ir „A-tail“ reagentai, kad būtų gauti paruošti dsDNR fragmentai 5' fosforilintu, 3'-dA užsibaigiančiu galu;
3. adapterių ligavimui, kai dsDNR adapteriai su 3'-dTMP iškyšomis liguojami su molekulėmis, turinčiomis 3-dA galus;
4. bibliotekos amplifikavimui (neprivaloma), kai naudojama didelio tikslumo PGR amplifikuoti fragmentus, kurių abiejuose galuose yra atitinkamos adapterio sekos.

Rinkinyje yra vienos koncentracijos buferis ir vienas fermentų mišinys fermentinei fragmentacijai bei kiekvienam iš dviejų bibliotekos sudarymo etapų; tačiau jame nėra adapterių ir magnetinių dalelių, kurių reikia valymui po adapterių ligavimo ir bibliotekos amplifikavimo. *KAPA Pure* magnetinės dalelės ir *KAPA* adapteriai parduodami atskirai.

Kad būtų užtikrintas maksimalus DNR sekos vienodumas, nepaprastai svarbu kiek įmanoma sumažinti bibliotekos amplifikavimo paklaidas. *KAPA HiFi* DNR polimerazė yra skirta nuo sekos GC/AT sankauptų nepriklausomo didelio tikslumo PGR ir yra pirmo pasirinkimo reagentas NGS bibliotekos amplifikavimui.^{1, 2, 3, 4} *KAPA HyperPlus* rinkiniuose yra *KAPA HiFi HotStart ReadyMix* (2X), paruoštas naudoti PGR mišinys, kuriam yra visi bibliotekos amplifikacijai reikalingi reagentai, išskyrus pradmenis ir DNR mėginį. Rinkiniuose taip pat yra bibliotekos amplifikavimo pradmenų mišinys (10X), skirtas didelio efektyvumo *Illumina* bibliotekų amplifikavimui, šalia kurių yra adapteriai su specifinėmis P5 ir P7 sekomis. Procesams, kuriuose nenaudojama PGR, siūlomi rinkiniai be amplifikacijos reagentų (KK8511, KK8513, KK8515). Juos galima naudoti kartu su *KAPA HiFi* realaus laiko bibliotekos amplifikavimo rinkiniais (KK2701, KK2702) ir tiksliau kontroliuoti visą bibliotekos amplifikavimo procesą.

1. Oyola, S. O., et al., *BMC Genomics* 13, 1 (2012).
2. Quail, M. A., et al., *Nature Methods* 9, 10 (2012).
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4. Ross, M. G., et al., *Genome Biology* 14, R51 (2013).

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Vertėja


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