

KAPA EvoPlusV2 Kits: every breakthrough begins with brilliant sample prep.

The **KAPA EvoPlus V2 Kits** offer improved fragmentation performance, insensitivity to fragmentation inhibitors, better conversion efficiency and reduced sequencing artefacts* through a streamlined and fully automatable workflow. This upgraded enzymatic fragmentation and library prep solution enables researchers to achieve higher confidence with increased sequencing efficiency.

KAPA EvoPlus V2 Kits offer a complete library preparation solution when combined with **KAPA Adapters** and **KAPA HyperPure Beads** (sold separately) and a complete target enrichment solution when combined with **KAPA HyperCap** or **KAPA HyperPETE workflows**. The kits are compatible with the Illumina sequencing platform and have been qualified with **automation methods**.

Benefits of the KAPA EvoPlus V2 Workflow*

Simplified and streamlined workflow

Tunable and trusted fragmentation

Exceptional library yields and sequencing quality

Enable superior performance and sequencing efficiency

Simplified, streamlined and automatable workflow with ReadyMix reagents, available in tube and plated format to increase efficiency and convenience

Compatible with a wide range of sample types, buffers and inputs and flexible with respect to fragment size, adapter design and library amplification

Designed to provide increased library conversion efficiency with the KAPA EvoT4 DNA Ligase in the Ligation ReadyMix, enabling higher sensitivity and more confident variant detection

Drastically reduced sequencing artefacts, with insensitivity to inhibitors, fully tunable fragmentation and improved library prep performance

Constantly evolving, efficient, and complete solutions



Sample-in

Nucleic Acid Extraction



Sample Quantification / QC



Library Preparation



Target Enrichment



Library Quantification



Sequencing Library





Sequencing Secondary Analysis Insights

KAPA EvoPlus V2 Kit

Simplified and streamlined workflow*

The **KAPA EvoPlus V2 Kit** provides a simplified and streamlined workflow to remove the complexities and risk for human error by providing a **trusted enzymatic fragmentation** solution.

- Streamlined library prep with combined Fragmentation and A-tailing step
- ReadyMix formulations, therefore fewer reagents and hands-on-time
- Tube and plated format increase efficiency and convenience
- Manual and automation* friendly protocol
- Reduces complexity of workflow and provides greater peace of mind
- Validated with the KAPA HyperCap Workflow and KAPA HyperPETE Workflow

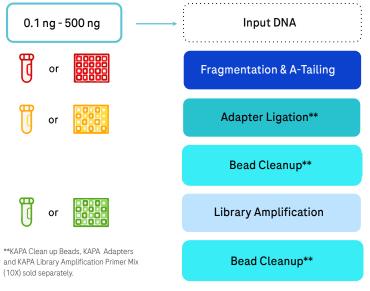


Figure 1: The KAPA EvoPlus V2 Workflow.

Tunable and trusted fragmentation

- Library insert sizes **adjustable** by varying fragmentation time
- Reproducible insert sizes across a range of GC content and DNA input amounts
- No impact to fragmentation—insensitive to EDTA (up to 2 mM), as well as numerous other inhibitors

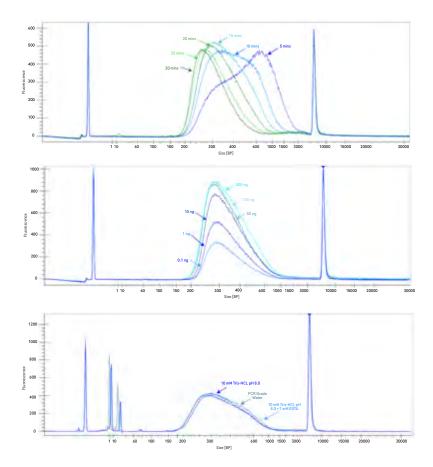


Figure 2: The KAPA EvoPlus V2 chemistry enables tunable enzymatic fragmentation. Human genomic DNA (100 ng) was fragmented at 37°C for different periods of time (5 – 30 min) to achieve mode library insert sizes ranging from approximately 250 – 1000 bp. The KAPA EvoPlus V2 workflow was completed without any size selection using full-length adapters (KAPA UDI Adapters) and 3 cycles of amplification to enable visualisation. Electropherograms were generated with LabChip GX Touch NGS 3K Assay.

Figure 3: KAPA EvoPlus V2 chemistry enables reproducible insert sizes across a range of DNA input amounts. 0.1 ng - 500 ng of high-quality human genomic DNA eluted in 10 mM Tris-HCl, pH 8.0 was fragmented for 25 minutes and used to prepare libraries with KAPA Universal Adapters at the recommended adapter:insert molar ratio following the KAPA EvoPlus V2 Kit Instructions for Use. Libraries were amplified for various cycles dependent on DNA input to enable visualization. Electropherograms were generated with LabChip GX Touch NGS 3K Assay.

Figure 4: The KAPA EvoPlus V2 chemistry enables reproducible enzymatic fragmentation across DNA diluted in different buffer types. 100 ng of high-quality human genomic DNA eluted in 10 mM Tris-HCl, pH 8.0, 10 mM Tris-HCl, pH 8.0 + 1 mM EDTA or PCR grade water was fragmented for 15 minutes and used to prepare libraries with KAPA UDI Adapters at the recommended adapter: insert molar ratio following the KAPA EvoPlus V2 Kit Instructions for Use. Libraries were amplified for 3 cycles to enable visualization. Electropherograms were generated with LabChip GX Touch NGS 3K Assay.

Exceptional library yields and sequencing quality

- · Achieve higher library yields across a range of input DNA and sample types
- Fewer amplification cycles for downstream processing result in lower duplication rates and higher sequence coverage
- Achieve successful library construction with biologically relevant samples and PCR-free workflows (from as little as 50 ng)

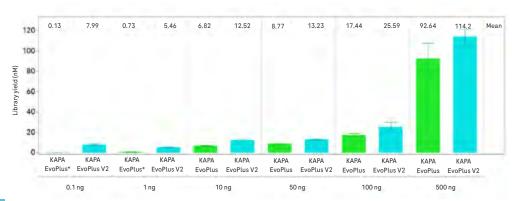


Figure 5: KAPA EvoPlus V2 chemistry enables high library conversion across a range of input DNA 0.1 ng - 500 ng of high-quality human genomic DNA was fragmented for 15 minutes and used to prepare libraries with KAPA Universal Adapters with KAPA UDI Primer Mixes at the recommended adapter: insert molar ratio following the KAPA EvoPlus and KAPA EvoPlus V2 Kit Instructions for Use. *Nonvalidated input (outside of the input range) of KAPA EvoPlus Kit - optimized cycle number for KAPA EvoPlus V2 Kit inputs used.

Enable superior performance and sequencing efficiency

- KAPA EvoPlus V2 Kits deliver high performing enzymatic fragmentation without drawbacks
- Improved sequencing metrics allow higher confidence in data due to reduction in sequencing artefacts

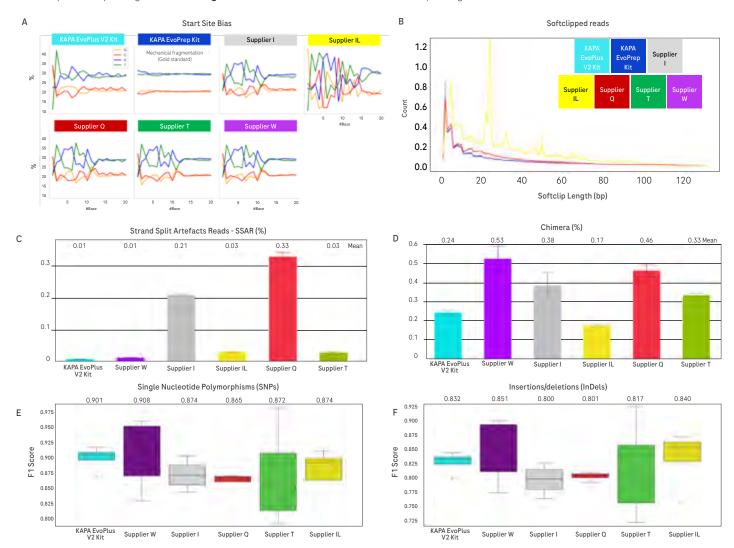


Figure 6. Reduction in sequencing artefacts observed in human WGS libraries prepared with the KAPA EvoPlus V2 Kit. PCR-free whole genome libraries were prepared using 100 ng of human genomic DNA (NA12878) with the KAPA EvoPlus V2 Kit, Supplier I, Supplier Q, Supplier T and Supplier W, following each supplier's instructions for use. (A) The KAPA EvoPrep Kit was included to present gold-standard¹ (mechanical fragmentation). The KAPA EvoPlus V2 Kit had the least start site bias compared to other Suppliers, resulting in higher data confidence², with the most start site bias associated with Supplier IL. (B) The KAPA EvoPlus V2 Kit had the lowest count of softclipped reads compared to other Suppliers, with Supplier IL having the highest count of softclipped reads. (C) The KAPA EvoPlus V2 Kit had the lowest percentage of SSARs present compared to other Suppliers, with Supplier Q having the highest percentage of SSARs present. SSARs represent chimeric reads that appear to be derived from non-contiguous portions of the genome³. (D) The KAPA EvoPlus V2 Kit had a lower percentage of Chimeras present, resulting in higher data confidence⁴ compared to Supplier I, Supplier Q, Supplier T and Supplier W. (E) and (F) KAPA EvoPlus V2 Kit had consistent, high sensitivity and specificity of detecting known variants (SNPs and InDels) compared to other suppliers.

Challenging the mechanical fragmentation status-quo

- Witness higher sequencing efficiency by higher specificity and higher duplex molecule recovery
- · Achieve higher result confidence by fewer artefacts compared to mechanical fragmentation library prep kits

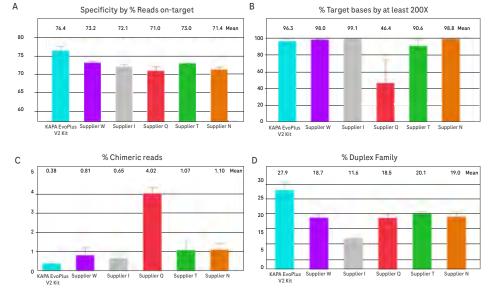


Figure 7. Improved sequencing performance in target capture workflow utilizing FFPET DNA 50 ng of low quality FFPET DNA was used to prepare triplicate libraries with the KAPA EvoPlus V2 Kit and mechanical fragmentation kits of Supplier I, Supplier N, Supplier Q, Supplier T and Supplier W, following each supplier's instructions for use. Libraries were enriched with the KAPA HyperCap Oncology Panel (214 Kb), following the KAPA HyperCap FFPET Evolved workflow instructions. (A) The KAPA EvoPlus V2 Kit had one of the highest percentage of reads on-target compared to mechanical fragmentation suppliers and (B) very high percentage of target bases covered by at least 200X compared to mechanical fragmentation suppliers, thereby showcasing the optimal utilization of sequencing throughput. (C) The KAPA EvoPlus V2 Kit had the lowest percentage of Chimeras present and (D) the highest duplex recovery, resulting in higher data confidence⁴ compared to mechanical fragmentation suppliers (Supplier I, Supplier N, Supplier Q, Supplier T and Supplier W).

Ordering information

| Roche cat. no. | Description | Pack size | Roche cat. no. | Description | Pack size |
|----------------|--|-----------|----------------|--|-----------|
| 09420037001* | KAPA EvoPlus V2 Kit (24rxn) | 24 rxn | 09420398001 | KAPA HiFi HS RM (9.6ml) | 9.6 mL |
| 09420053001* | KAPA EvoPlus V2 Kit (96rxn) | 96 rxn | 09420444001 | KAPA HiFi HS RM 96 well plate (96rxn) | 96 rxn |
| 09420339001* | KAPA EvoPlus V2 Kit (384rxn) | 384 rxn | 09420410001 | KAPA Library Amp Primer Mix (384 rxn) | 384 rxn |
| 09420428001* | KAPA EvoPlus V2 Kit, plated format (96rxn) | 96 rxn | 09420479001 | KAPA Library Amp Primer Mix 96-well plate (96rxn) | 96 rxn |
| 09420045001** | KAPA EvoPlus V2 Kit, PCR-free (24rxn) | 24 rxn | 10212284702*** | KAPA EvoPlus V2 Kit + Lib Amp Primers (24rxn) | 24 rxn |
| 09420304001** | KAPA EvoPlus V2 Kit, PCR-free (96rxn) | 96 rxn | 10212292702*** | KAPA EvoPlus V2 Kit + Lib Amp Primers (96rxn) | 96 rxn |
| 09420371001** | KAPA EvoPlus V2 Kit, PCR-free (384rxn) | 384 rxn | 10212306702*** | KAPA EvoPlus V2 Kit + Lib Amp Primers (384rxn) | 384 rxn |
| 09420436001** | KAPA EvoPlus V2 Kit, PCR-free, plated format (96rxn) | 96 rxn | 10212314702*** | KAPA EvoPlus V2 Kit+Lib Amp Primers (96 rxn plate) | 96 rxn |

KAPA Library Amplification Primer Mix (10X) not included.

- 1. Deurenberg et al. (2017). Application of next generation sequencing in clinical microbiology and infection prevention. Journal of Biotechnology 243, 16-24.
- 2. McNulty, et al. (2020). Impact of reducing DNA input on next-generation sequencing library complexity and variant detection. The journal of Molecular Diagnostics, Volume 22, Issue 5, May, Pages 720-727.
- 3. Haile, et al. (2019), Sources of erroneous sequences and artifact chimeric reads in next generation sequencing of genomic DNA from formalin-fixed paraffin-embedded samples. Nucleic Acids Research, 47,2.
- 4. Chen, et al. (2024). Characterization and mitigation of artifacts derived from NGS library preparation due to structure-specific sequences in the human genome. BMC Genomics 25:227.



Learn more at sequencing.roche.com

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^{**}KAPA HiFi HS RM and KAPA Library Amplification Primer Mix (10X) not included.

^{***} Virtual kits.