KAPA EvoPlus V2 Kits Guide to Success

Streamlined sample prep workflow using the next evolved generation of KAPA DNA Library Prep Reagents.

Optimize amplification cycles for your downstream application

Input DNA	Number of cycles to generate ≥4 nM* of library (if using KAPA UDI Adapters)	Number of cycles to generate ≥4 nM* of library (if using truncated KAPA Universal Adapter & KAPA UDI Primer Mixes)
0.1 ng	11 - 13	12 - 13
1 ng	7 - 9	8 - 9
10 ng	3 - 5	4 - 5
50 – 500 ng	0 (PCR free)	3 - 4

* The number of cycles needed depends on the specific adapter and amplification primer design, as well as input type and quality

1 INPUT DNA

Setup is at 4°C

2 ENZYMATIC FRAGMENTATION & A-TAILING

Variable time (see below)

Incubate at 37°C

30 min

Followed by 55°C

3 ADAPTER LIGATION

15 min

Incubate at 20°C

4 POST-LIGATION BEAD CLEANUP

0.8X if using KAPA Universal Adapter & UDI Primer Mixes

0.6X if using KAPA full length adapters

5 LIBRARY AMPLIFICATION

6 1X POST-AMP BEAD CLEANUP

7 SECOND POST-AMP CLEANUP

Only if using KAPA Universal Adapter & KAPA UDI Primer Mixes

Or using less than 1 ng with KAPA UDI Adapters

ReadyMixes are already prepared for all EvoPlus V2 Library steps

PCR-free Whole Genome Sequencing

Proceed to target enrichment or Whole Genome Sequencing
Visit us at sequencing.roche.com/support

Is my library of sufficient quality and quantity to proceed?
Assess both the size and concentration of the library with an appropriate quantification method.

YES → **Sequence the library**

NO → **Visit us at** sequencing.roche.com/support

How much DNA do I need?

Application	Sample type	Input
WGS	High quality gDNA	0.1 ng – 500 ng
	Low quality FFPET-derived DNA	≥ 50 ng*
WGS (PCR-free)	High quality gDNA	≥ 50 ng (no-SS)** 500 ng (with SS)**
Targeted Sequencing	High quality gDNA	100 ng
	Low quality FFPET-derived DNA	10 ng - 50 ng

* Reach out to Technical Support for possible workflow modifications when using this sample type.
** SS = double-sided size selection; a requirement when performing WGS on patterned flow cells but may result in sample losses of 60 – 95%, irrespective of whether a bead- or gel-based technique is used. For PCR-free workflows, due to the inherent sample losses, performing double-sided size selection with inputs <500 ng (into library prep) is not recommended.

How long should I fragment my DNA for?

- Mode and size distribution of DNA is controlled by fragmentation time and temperature.
- Try a range of fragmentation times to determine optimal insert size.

Estimated Insert Size*	Fragmentation time at 37°C
180 bp	30 min
200 bp	25 min
250 bp	20 min
300 bp	15 min
450 bp	10 min
500 bp	5 min

* Insert sizes (without adapter) observed upon fragmentation of 100 ng of high quality human DNA12878 (Coriell Institute of Biomedical Research). Size variation may be observed, depending on DNA type, DNA input and DNA elution buffers. We recommend optimizing the fragmentation time with a non-precious sample.

How much adapter do I need?

Adapter concentration affects ligation efficiency, as well as adapter and adapter-dimer carry-over during the post-ligation cleanup.

Input DNA	Recommended KAPA UDI Adapter stock concentration	Recommended KAPA Universal Adapter stock concentration
0.1 ng	0.6 µM	0.6 µM
1 ng		
10 ng	6 µM	15 µM
≥ 10 ng	15 µM