KAPA HYPERPLUS KIT
Guide to Success

Roche

KAPA HyperPlus workflow: single-tube fragmentation and library preparation workflow in less than 3 hours

Contact Technical Support at **sequencing.roche.com/support**

1 INPUT DNA

setup is at 4˚C

2 ENZYMATIC FRAGMENTATION

variable time *(see below)*

incubate at 37˚C

assess size after amplification

KEEP GOING

place samples at 4˚C

3 END REPAIR AND A-TAILING

incubate at 65˚C

30 min

4 ADAPTER LIGATION

incubate at 20˚C

15 min

5 0.8X POST-LIGATION CLEANUP

optional size selection

6 LIBRARY AMPLIFICATION

7 POST- AMP CLEANUP

Full-Length Adapters: Follow 1X post-amp cleanup

Truncated Adapters: Follow modified post-amp cleanup

Got EDTA?

- The enzymatic fragmentation reaction is sensitive to EDTA.
- The best strategy is to remove EDTA by means of a cleanup step before fragmentation.
- If the input DNA contains EDTA, please see Table 3 Instructions for Use (IfU) of the KAPA HyperPlus Kit.

How much DNA do I need?

Application	Sample type	Input
WGS	High quality gDNA	50 ng – 1 µg
	Low quality FFPET-derived DNA	≥50 ng*
WGS (PCR-free)	High quality gDNA	≥50 ng (no SS)** ≥200 ng (with SS)**
Targeted Sequencing	High quality gDNA	≥100 ng
	Low quality FFPET-derived DNA	≥100 ng*
	Long amplicons	≥1 ng
RNA-Seq	Full-length/ unfragmented cDNA	≥1 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 <200 ng is not recommended.

Get to chopping.

- Mode and size distribution of DNA is controlled by fragmentation time and temperature.
- Try a range of fragmentation times to determine optimal insert size.
- For ease of sample processing, place samples with the longest fragmentation time in the thermal cycler first. Add samples with shorter fragmentation times at appropriate intervals.

Mode fragment length	Incubation time at 37°C*	Optimization range
600 bp	5 min	3 – 10 min
350 bp	10 min	5 – 20 min
200 bp	20 min	10 – 25 min
150 bp	30 min	20 – 40 min

* These parameters are a good starting point for high-quality genomic DNA. Please refer to Appendix 2: Optimization of Fragmentation Parameters of the TDS for guidelines on how to optimize fragmentation time and temperature, if needed.

It's not a typo!

- Ensure that the correct volume of KAPA Frag Buffer (5 µL) and KAPA Frag Enzyme (10 µL) is added to each reaction.

Component	Volume
Double-stranded DNA *(with KAPA Frag Conditioning Solution, if needed)*	35 µL
KAPA Frag Buffer (10X)	5 µL
KAPA Frag Enzyme	10 µL
Total volume	**50 µL**

Which End Repair and A-Tailing Enzyme Mix to use?

- The formulation of the End Repair and A-Tailing (ERAT) Enzyme Mix has been enhanced to improve performance for more sensitive assays.
- The user must choose which ERAT Enzyme Mix to use:

Enzyme name	Cap color	When to use
HyperPrep ERAT Enzyme Mix	Purple	Existing, validated workflows
HyperPlus ERAT Enzyme Mix	Orange	New workflows; more sensitive applications

ERAT = End Repair and A-Tailing

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	Adapter stock concentration	Adapter:insert molar ratio
1 µg	15 µM	10:1
500 ng	15 µM	20:1
250 ng	15 µM	40:1
100 ng	15 µM	100:1
50 ng	15 µM	200:1
25 ng	7.5 µM	200:1
10 ng	3 µM	200:1
5 ng	1.5 µM	200:1
2.5 ng	750 nM	200:1
1 ng	300 nM	200:1

How many cycles will it take?

- Recommended number of amplification cycles to generate 4 nM of amplified DNA when using KAPA Universal Adapter & KAPA UDI Primer Mixes.

Input amount	Number of amplification cycles for WGS to achieve 4 nM* (if using truncated Universal Adapter & KAPA UDI Primer Mixes)
500 ng*	3 - 4 cycles
250 ng	3 - 5 cycles
10 ng	5 – 7 cycles
1 ng	10 – 12 cycles

Note: When using incomplete, or truncated, adapters (such as KAPA Universal Adapter & KAPA UDI Primer Mixes), a minimum number of amplification cycles (3) are required to complete adapter sequences for the next step in the process (target capture or sequencing), irrespective of whether a sufficient amount of library is available after ligation. The number of cycles needed depends on the specific adapter, downstream application and amplification primer design.

* Based on sequencing recommendations, 4 nM is the minimum starting concentration to proceed with sequencing. Users requiring concentrations >4 nM can adjust the number of amplification cycles in 2 cycle increments until the target concentration is achieved. This may require optimization. Note: increasing cycle numbers ultimately decreases the library complexity by increasing the duplication rate.

Work smarter, not harder.

- Determine how much final library material is required for the downstream application (e.g., capture, sequencing).

Input into library construction (Full length Adapters)	Number of cycles required to generate	
	100 ng library	1 µg library
1 µg	0	0 – 1
500 ng	0	2 – 3
250 ng	0 – 1	3 – 5
100 ng	0 – 2	5 – 6
50 ng	3 – 5	7 – 8
25 ng	5 – 6	8 – 10
10 ng	7 – 9	11 – 13
5 ng	9 – 11	13 – 14
2.5 ng	11 – 13	14 – 16
1 ng	13 – 15	17 – 19

Is my library sufficient?

- Assess both the size and concentration of the library with an appropriate quantification method.

YES

8 TARGET CAPTURE OR SEQUENCING

Success!
Order more KAPA HyperPlus Kits from a **local sales representative**.

NO

Do I have adapter-dimers?

NO

Visit **sequencing.roche.com/support**

YES

Repeat Step 7

Data on file. For Research Use Only. Not for use in diagnostic procedures.

input DNA | 4˚C | enzymatic fragmentation | 4˚C | end repair & A-tailing | adapter ligation | 0.8X post-ligation cleanup | optional size selection | library amplification | 1X post-amp cleanup | target capture or sequencing