Rapid Plus DNA Lib Prep Kit for Illumina V2

RK20255



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1.Introduction

The Rapid Plus DNA Lib Prep Kit for Illumina V2 (Cat. No. RK20255) inherits the high performance of the Rapid Plus DNA Lib Prep Kit and further improves the ligation efficiency at low input levels. It is a universal library construction kit.

This kit can construct DNA sequencing libraries from DNA samples ranging from 1 ng to 1000 ng, suitable for Illumina® high-throughput sequencing platforms. The kit includes a series of enzymes and reaction buffers required for library construction experiments, such as end repair of DNA samples, addition of dA, ligation of sequencing adapters, and library amplification, facilitating the rapid construction of libraries. The entire experimental process can be completed within 2-3 hours. It is applicable to various sample types, including normal genomes, cell-free DNA, FFPE DNA, and CHIP DNA. Additionally, this kit can also be used for PCR-Free library construction.

The components of the kit have undergone strict quality control, including tests for component contamination, functional validation, application scenario testing, and product batch-to-batch stability testing, among other procedures. This minimizes the residual pathogens to the greatest extent, making it suitable for pathogen detection.

2. Components

Table 1. Components supplied with this product

		Components	24 RXN	96 RXN
End Repair & dA Tailing		End Prep Buffer II	168 μL	672 μL
		End Prep Enzymes	72 μL	288 μL
		Damaged DNA Repair Enzymes II	72 μL	288 μL
Adapter Ligation		Ligation Buffer	720 μL	2880 μL
		Ligase Enzymes II	240 μL	960 μL
Amplification		Gloria Nova HS 2X PCR Mix for NGS	600 μL	2400 μL
		10X PCR Primers	120 μL	480 μL

3. Storage

Transportation and storage: the Rapid Plus DNA Lib Prep Kit for Illumina V2 must be stored at -15 °C to -25 °C. Because this kit is sensitive to temperature, dry ice or dry ice combined with ice packs should be used for long-distance transportation.

4. Applications

The Rapid Plus DNA Lib Prep Kit for Illumina V2 is suitable for next-generation DNA sequencing library construction. The main functional modules include end repair, 3’A-tailing, damaged DNA repair, adapter ligation, and PCR amplification. This kit is applicable to various sample types with starting amounts ranging from 1 ng to 1000 ng, including normal genomes, cell-free cfDNA and ctDNA, FFPE DNA, CHIP DNA, PCR products, cDNA products, etc.

In summary, the application scenarios of the Rapid Plus DNA Lib Prep Kit for Illumina V2 mainly include:

a. Whole-genome sequencing;
b. Exome sequencing and targeted sequencing, including Roche® NimbleGen™ SeqCap™ EZ, Agilent SureSelect, Illumina TruSeq®, IDT xGen™ Lockdown™ Probes, or other probe hybridization systems;
c. ChIP-seq, with input DNA being ChIP DNA;
d. RNA-seq, with input DNA being cDNA products;
e. Amplicon sequencing, with input DNA being PCR products;
f. Metagenomic sequencing;
g. cfDNA/ctDNA sequencing.

5. Additional Materials Required

5.1 Main Reagents and Instruments/Consumables

a. Anhydrous ethanol (80% ethanol should be prepared freshly when needed), nuclease-free water, Low-EDTA TE Buffer (ABclonal, Cat. No. RM20206)

b. PCR tubes, magnetic stand, pipette tips with filters, PCR thermal cycler, microcentrifuge, vortex mixer, single-channel pipette, and multi-channel pipette

c. AB Qubit dsDNA Quantitation Kit (ABclonal, Cat. No. RK30140 / RK30141), Agilent Technologies 2100 Bioanalyzer or other equivalent DNA quality control products

5.2 Magnetic Beads

AFTMag NGS DNA Clean Beads (ABclonal, Cat. No. RK20257)

5.3 Sequencing adapters for Illumina® Platform

a. Truncated adapters: Cat. No. RK21622 - RK21627

b. Full-Length adapter: Cat. No. RK21706 - RK21709

For specific information, please refer to the summary table of compatible adapter types for the Rapid Plus DNA Lib Prep Kit for Illumina V2 in the **Appendix Table**.

6. Notes

6.1 Input DNA

6.1.1. This kit is compatible with DNA that has been fragmented by mechanical shearing and enzymatic digestion.

6.1.2. The term "Input DNA" referred to in the following text specifically denotes the DNA that is introduced into the end repair/dA tailing step.

6.1.3. The kit is compatible with Input DNA in the range of 1 ng to 1000 ng. High-quality Input DNA with an A260/A280 ratio of 1.8-2.0 should be used whenever possible.

6.1.4. If high concentrations of metal ion chelators or other salts are introduced during the preparation of Input DNA, it may affect the reaction efficiency of the end repair/dA tailing step. It is recommended to purify or sort the DNA fragments using magnetic beads after fragmentation.

6.1.5. When performing DNA fragmentation using a mechanical method, please dilute the DNA in TE Buffer for fragmentation. Do not fragment the DNA in sterile ultrapure water.

6.1.6. If the DNA sample has been purified or sorted after mechanical shearing, its concentration should be measured again. This will be used as the Input DNA amount. Otherwise, it may result in low library yield.

6.1.7. When performing fragmentation using an enzymatic method and the product is not purified or sorted but directly used for library construction, please ensure that the Stop Buffer does not contain an excess of metal ion chelators. If the condition is not met, the fragmented product can first be purified or length-sorted and then

dissolved in TE Buffer or sterile ultrapure water (≤50 μL) before proceeding with library construction.

6.2 Damaged DNA Repair Enzymes II

The Damaged DNA Repair Enzymes II included in this library preparation kit has a strong ability to repair damaged DNA. The main types of damage it addresses include nicks, gaps, oxidized bases, damaged or blocked 3' ends, AP sites (apurinic/apyrimidinic sites), and uracil bases. For challenging or degraded samples such as FFPE DNA, this repair system can significantly improve DNA library yield and overall success rate of library construction. However, in some FFPE samples, the use of DDRE may lead to a slight increase in chimeric read rates. Whether to use this repair step can be determined based on the specific sample and experimental requirements.

6.3 Adapter

6.3.1. ABclonal offers both Full-Length adapter kits and Truncated adapter kits, details of which can be found in the attached table. Customers can make their selection based on experimental requirements.

6.3.2. When constructing PCR-Free libraries, it is necessary to use Full-Length adapters with full-length indexes in the adapter ligation step. After the ligation, the excess adapters should be removed from the adapter ligated product before sequencing.

6.3.3. This kit is compatible with various non-indexed, single-end indexed and dual-end indexed adapters used in conventional application systems such as Illumina TruSeq, Roche NimbleGen SeqCap EZ, and Agilent SureSelect.

6.3.4. The quality and concentration of the adapters directly affect the ligation efficiency and library yield. An excessive amount of adapters may lead to the formation of more adapter dimers, while an insufficient amount may reduce the ligation efficiency and library yield. **Table 3** lists the recommended dilution of adapters for different input DNA amounts when using this kit.

Table 3. Recommended adapter concentrations

Input DNA	Dilution Ratio	Working Concentration
1 μg-50 ng	Undiluted	15 μM
49 ng-25 ng	1:2	7.5 μM
24 ng-10 ng	1:5	3 μM
9 ng-5 ng	1:10	1.5 μM
< 5 ng	1:20	0.75 μM

6.4 Clean Beads

6.4.1 Magnetic beads should be equilibrated to room temperature before use; otherwise, it will lead to a decrease in yield and poor separation efficiency.

6.4.2 Magnetic beads should be thoroughly vortexed and mixed before each use.

6.4.3 When transferring the supernatant, do not aspirate the magnetic beads, as even a small amount of residual beads will affect the quality of the subsequent library.

6.4.4 The 80% ethanol used for magnetic bead washing should be prepared freshly before use; otherwise, it will affect the recovery efficiency.

6.4.5 Before elution of the product, the magnetic beads should be dried at room temperature. Inadequate drying can easily cause residual anhydrous ethanol to affect subsequent reactions; excessive drying, on the other hand, will cause the magnetic beads to crack and thus reduce the purification yield. Under normal circumstances, drying at room temperature for 3-5 minutes is sufficient to thoroughly dry the magnetic beads.

6.4.6 The typical purification condition for the ligation product is 0.8× (110 μL of ligation product, 88 μL of magnetic beads), which is applicable to the vast majority of situations. If the Insert Size is relatively small, you can try changing the purification condition of the ligation product to 1× (110 μL of ligation product, 110 μL of magnetic beads). To obtain a library with a longer Insert Size, you can appropriately reduce the amount of magnetic beads used to decrease the proportion of small fragments. However, this adjustment can only roughly change the position of the library's main peak. If you need to accurately control the length distribution of the library, length selection can be performed after this purification step. The detailed steps for fragment selection are provided in the appendix.

6.4.7 The DNA fragment length selection step can be carried out before end-repairing/dA-tailing, after adapter ligation, or after library amplification. When the Input DNA quality is ≥50 ng, fragment selection can be performed after adapter ligation; if the Input DNA quality is <50 ng, it is recommended to perform selection after library amplification.

6.4.8 If the quality control results show severe contamination of Adapter

or Adapter Dimer in the purified product, a second magnetic bead purification can be attempted: Dilute the volume of the first purified product to 50 μL with sterile ultrapure water, and then add 50 μL of magnetic beads (1 ×) for the second purification. This can significantly reduce the residual Adapter or Adapter Dimer, especially when constructing PCR-Free libraries. Sometimes, it may also be necessary to reduce the amount of Adapter used in order to completely eliminate the residual Adapter or Adapter Dimer.

6.5 Amplification

6.5.1 It is recommended to use Qubit to quantify Input DNA; a higher amount of input DNA can improve the efficiency of DNA ligation, so it is suggested to increase the amount of Input DNA; if the starting DNA contains enzyme inhibitors, it is recommended to purify it with 2.2× magnetic beads before library construction.

6.5.2 Extending the ligation reaction time can improve the efficiency of adapter ligation. You can try appropriately extending the ligation time to 25 min - 4 hr, or performing the ligation overnight at 4℃/ 16℃.

6.5.3 A higher primer concentration can increase library yield and reduce large DNA fragments. You can increase the primer concentration, but the adjustment should be controlled within the range of 5 - 20 μM.

6.5.4 If the amount of Input DNA is ≥ 100 ng, you can divide the purified ligation product into equal parts and amplify it using two 50-μL PCR systems, which can increase library yield and reduce large DNA fragments. For low-abundance DNA or difficult/degraded DNA samples, it is recommended to appropriately increase the number

of PCR cycles by 1 - 3.

6.5.5 An appropriate magnetic bead purification ratio can ensure a stable library yield. You can try increasing the magnetic bead ratio to above 1.0× to reduce the loss of short fragments below 200 bp. At the same time, prevent excessive magnetic bead ratio from causing adapter dimer formation. If the library contains adapter dimers or primer residues, it is recommended to re-purify and recover the DNA with 1.0× magnetic beads.

6.6 Reagent Preparation

6.6.1 Before use, ensure that End Prep Buffer II, Ligation Buffer, Gloria Nova HS 2X PCR Mix for NGS, and 10X ILM PCR Primers are completely and thoroughly dissolved.

6.6.2 The reagent components in the kit can be fully dissolved at room temperature. After mixing and a brief centrifugation, place them on ice for later use.

6.6.3 Some components of this kit are relatively viscous, especially the Ligation Buffer. Extra care should be taken during pipetting operations, and it is recommended to aspirate or mix the solution or components at a slower speed. After using the product components, please store them as soon as possible at -15 to -25℃.

6.6.4 End Prep Buffer II should be stored away from light as much as possible. The buffer has a yellowish color due to the presence of the NAD+ coenzyme factor, which does not affect its use.

7.Protocol

7.1 End Repair & dA Tailing

7.1.1 Prepare the following reaction system in a sterile PCR tube:

Table 4. End Repair & dA Tailing Reaction Setup (per sample)

	Component	Volume
	Input DNA	X μL
●	End Prep Buffer II	7 μL
●	End Prep Enzymes	3 μL
○	Nuclease-Free Water	Up to 60 μL
	Total volume	**60 μL**

Note: For degraded samples such as FFPE, you may try adding 3 μL of Damaged DNA Repair Enzymes II (while maintaining a total reaction volume of 60 μL).

7.1.2 Mix the reaction system thoroughly.

7.1.3 Place the PCR tube into the PCR machine. The reaction program is shown in **Table 5**, and the PCR machine's heated lid should be set to 75℃.

Table 5. Thermal cycler program for End Repair & dA Tailing

Temperature	Time
20℃ *	30 min
65℃	30 min
4℃	∞

Note: If Damaged DNA Repair Enzymes II is added, the reaction temperature should be adjusted from 20 ℃ to 30 ℃.

7.2 Adapter Ligation

7.2.1 Remove the required magnetic beads from the 4℃ refrigerator in advance and equilibrate them to room temperature. Vortex the beads to mix thoroughly. Allow them to equilibrate at room temperature for at least 30 minutes.

7.2.2 Dilute the Working Adapter according to **Table 3** using Low-EDTA TE Buffer.

7.2.3 Prepare the reaction system shown in **Table 6** on ice. Nuclease-Free Water, Ligation Buffer, and Ligase Enzymes II can be combined into a premix, but the Working Adapter should be added separately. After preparing the ligation reaction system, mix thoroughly and centrifuge.

Table 6. Adapter Ligation Reaction Setup (per sample)

	Component	Volume
	End Prep Reaction Mix（**Step 7.1.3**）	60 μL
	Nuclease-Free Water	5 μL
	Ligation Buffer	30 μL
	Ligase Enzymes II	10 μL
	Working Adapter（**Table 3**）	5 μL
	Total volume	**110 μL**

7.2.4 Place the PCR tube into the PCR machine. The reaction program is shown in **Table 7**. Do not use the heated lid on the PCR machine.

Table 7. Thermal cycler program for Adapter Ligation Reaction

Temperature	Time
20℃	15 min
4℃	∞

7.2.5 If there is a strict requirement for the library size, size selection can be performed after the adapter ligation reaction. The size selection protocol can be found in the appendix. If size selection is not required, proceed directly to **Step 7.2.6**.

7.2.6 In the 110 μL ligation reaction system, add 88 μL (0.8×) AFTMag NGS DNA Clean Beads to each sample. Vortex to mix thoroughly and incubate at room temperature for 5 minutes.

7.2.7 Place the PCR tube on a magnetic rack and allow it to stand for 2 minutes. Once the solution is clear, remove the supernatant without disturbing the magnetic beads.

7.2.8 Add 200 μL of 80% ethanol to wash the magnetic beads. After incubating for 30 seconds, remove the supernatant.

7.2.9 Repeat **step 7.2.8**.

7.2.10 Keep the PCR tube on the magnetic rack and use a 10 μL pipette to remove any residual ethanol from the bottom of the tube. Open the tube cap and allow it to dry until all ethanol is removed.

7.2.11 Remove the PCR tube from the magnetic rack and resuspend the magnetic beads in 21 μL of Nuclease-Free Water. Allow it to stand at room temperature for 1 minute.

7.2.12 Place the PCR tube on the magnetic rack and allow it to stand for 2 minutes. Transfer 20 μL of the supernatant to a new PCR tube.

Safety Pause Point: The purified ligated product can be temporarily stored at 4°C for up to 1 week. For long-term storage, it should be kept at -20°C to avoid repeated freeze-thaw cycles.

7.3 Amplification

This kit can use full-length or truncated adapters suitable for Illumina and MGI platforms. Depending on the type of adapter, choose the library

amplification system from **Table 8**, **Table 9**. The adapter types and related product numbers are listed in the **Appendix**.

7.3.1 Prepare the library amplification system according to the type of adapter:

Table 8. Amplification Reaction Setup for Illumina (Full-Length)

	Component	Volume
	Adapter-ligated DNA	20 μL
●	Gloria Nova HS 2X PCR Mix for NGS	25 μL
●	10X ILM PCR Primers	5 μL
	Total volume	**50 μL**

Table 9. Amplification Reaction Setup for Illumina (Truncated)

	Component	Volume
	Adapter-ligated DNA	20 μL
●	Gloria Nova HS 2X PCR Mix for NGS	25 μL
○	UDI Primer xxx	5 μL
	Total volume	**50 μL**

7.3.2 Mix thoroughly and centrifuge briefly.

7.3.3 Place the PCR tube in the PCR machine. Refer to **Table 10** for the reaction program and **Table 11** for the recommended number of library amplification cycles. Set the PCR machine's heated lid to 105℃.

Table 10. Thermal cycler program for Library Amplification

Temperature	Time	Cycles
98℃	1 min	1
98℃	10 s	2-19 PCR Cycles
60℃	30 s	
72℃	30 s	
72℃	1 min	1
4℃	∞	1

Table 11. Recommended cycle numbers

Input DNA（ng）	Recommended PCR cycles for 1 μg library yield (Illumina)
1000	2-3
500	3-4
250	4-5
100	5-6
50	6-7
25	7-9
10	9-11
1	13-15
0.1	17-19

*Note:

1. For FFPE samples, increase the number of PCR cycles by 1-3.

2. If size selection is performed after adapter ligation, it is recommended to add 2 additional PCR cycles.

7.3.4 Add 50 μL (1×) AFTMag NGS DNA Clean Beads to the amplification product, vortex to mix thoroughly, and incubate at room temperature for 5 minutes.

7.3.5 Place the PCR tube on a magnetic rack and let it stand for 2 minutes. Once the solution becomes clear, carefully remove the supernatant without disturbing the beads.

7.3.6 Add 200 μL of 80% ethanol to wash the beads. After incubating for 30 seconds, remove the supernatant.

7.3.7 Repeat **step 7.3.6**.

7.3.8 Keep the PCR tube on the magnetic rack. Use a 10 μL pipette to remove any residual ethanol at the bottom of the tube. Open the tube cap and allow it to air-dry until no ethanol remains.

7.3.9 Remove the PCR tube from the magnetic rack. Add 31 μL of Nuclease-Free Water to resuspend the beads and let it stand at room temperature for 1 minute.

7.3.10 Place the tube back on the magnetic rack for 2 minutes, then transfer 30 μL of the supernatant to a new PCR tube.

7.3.11 Store the library at -20°C for subsequent quality assessment.

Safe stopping point: The purified PCR product can be temporarily stored at 4°C for up to one week. For long-term storage, keep it at -20°C and avoid repeated freeze-thaw cycles.

8. Appendix

8.1 Size Selection

For size selection, after adapter ligation, perform a 1× magnetic bead purification first, then carry out two rounds of magnetic bead-based size selection in a 100 μL aqueous system from the purified sample. Using Illumina truncated adapter libraries as an example, **Table 12** provides recommended bead ratios for obtaining different insert sizes.

Table 12. Recommended bead ratios for obtaining different insert sizes

Insert Size (bp)	150	200	250	300	350	450
1st bead ratio（AX）	1 X	0.8 X	0.75 X	0.7 X	0.6 X	0.57 X
2nd bead ratio（BX）	0.5 X	0.5 X	0.2 X	0.15 X	0.2 X	0.15 X

Here's an example for obtaining a 300 bp insert size:

8.1.1 Allow the magnetic beads to equilibrate to room temperature, then vortex thoroughly to mix.

8.1.2 In the 110 μL post-ligation system (**Step 7.2.4**), add 110 μL (1×) AFTMag NGS DNA Clean Beads to each sample, vortex to mix, and incubate at room temperature for 5 minutes.

8.1.3 Place the PCR tube on a magnetic rack for 2 minutes. Once the solution becomes clear, carefully remove the supernatant without disturbing the beads.

8.1.4 Add 200 μL of 80% ethanol to wash the beads. After incubating for 30 seconds, remove the supernatant.

8.1.5 Repeat **step 8.1.4**.

8.1.6 Keep the PCR tube on the magnetic rack. Use a 10 μL pipette to remove any residual ethanol at the bottom of the tube. Open the

tube cap and allow it to air-dry until no ethanol remains.

8.1.7 Remove the PCR tube from the magnetic rack. Add 102 μL of Nuclease-Free Water to resuspend the beads and let it stand at room temperature for 1 minute.

8.1.8 Place the tube back on the magnetic rack for 2 minutes, then transfer 100 μL of the supernatant to a new PCR tube.

8.1.9 Add 70 μL (0.7×) AFTMag NGS DNA Clean Beads to the 100 μL purified aqueous system, mix well, and incubate at room temperature for 5 minutes.

8.1.10 Place the PCR tube on the magnetic rack for 2 minutes. Transfer the supernatant to a new PCR tube, being careful not to carry over any beads.

8.1.11 Add 15 μL (0.15×) AFTMag NGS DNA Clean Beads to the above supernatant, mix well, and incubate at room temperature for 5 minutes.

8.1.12 Place the sample on the magnetic rack for 2 minutes. Once the solution becomes clear, carefully remove the supernatant without disturbing the beads.

8.1.13 Add 200 μL of 80% ethanol to wash the beads. After incubating for 30 seconds, remove the supernatant.

8.1.14 Repeat **step 8.1.13**.

8.1.15 Keep the PCR tube on the magnetic rack. Use a 10 μL pipette to remove any residual ethanol at the bottom of the tube. Open the tube cap and allow it to air-dry until no ethanol remains.

8.1.16 Remove the PCR tube from the magnetic rack. Add 21 μL of Nuclease-Free Water to resuspend the beads and let it stand at room temperature for 1 minute.

8.1.17 Place the sample on the magnetic rack for 2 minutes, then transfer 20 μL of the supernatant to a new PCR tube for library amplification.

Safe stopping point: The purified ligation product can be temporarily stored at 4°C for up to one week. For long-term storage, keep it at -20°C and avoid repeated freeze-thaw cycles.

9.Appendix Table

Table 13. Summary of Compatible Illumina Adapters and Index Kits

Index type	Name	Cat.No
Illumina UDI (8-base)	Unique Dual Index for Illumina MiniSet (8 indices)	RK21622
	Unique Dual Index for Illumina MidiSet (24 indices)	RK21623
	Unique Dual Index for Illumina Set_A (48 indices)	RK21624
	Unique Dual Index for Illumina Set_B (48 indices)	RK21625
	Unique Dual Index for Illumina Set_C (48 indices)	RK21626
	Unique Dual Index for Illumina Set_D (48 indices)	RK21627
Illumina UDI/UMI(8-base)	Unique Dual Index (with UMI) for Illumina MidiSet (24 indices)	RK21701
	Unique Dual Index (with UMI) for Illumina Set_A (48 indices)	RK21702
	Unique Dual Index (with UMI) for Illumina Set_B (48 indices)	RK21703
Illumina UDI (8-base)	Unique Dual Index Adapter Plate for Illumina Set_A (UDI 1-96)	RK21706
	Unique Dual Index Adapter Plate for Illumina Set_B (UDI 97-192)	RK21707
	Unique Dual Index Adapter Plate for Illumina Set_C (UDI 193-288)	RK21708
	Unique Dual Index Adapter Plate for Illumina Set_D (UDI 289-384)	RK21709
Illumina UDI (10-base)	Truncated DNA Adapter Plate for Illumina Set A/B/C/D/E/F/G/H (10 nt, UDI Primers 1-768)	RK21731 - RK21738
Illumina UDI (10-base)	Truncated DNA Adapter Plate for Illumina Set I/J/K/L/M/N/O/P (10 nt, UDI Primers 769-1536)	RK21739 - RK21746

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