Rapid Plus DNA Lib Prep Kit for Illumina® V2

RK20255

(1 ng-1 μg Input DNA)

(Illumina® Compatible)

www.abclonal.com

Version：N16A25v2.2

Contents

1. Introduction

Rapid Plus DNA Lib Prep Kit for Illumina®V2 (Cat. # RK20255) is designed to provide up to 96 indexed libraries for high multiplexing capabilities on Illumina® NGS platforms. The Kit contains all enzymes and buffers required to convert a broad range of input amounts of DNA into high quality libraries for next-generation sequencing on Illumina® NGS platforms. Inputs of 1 ng to 1000 ng double-stranded DNA (dsDNA) are required for library preparation. The entire four-step workflow takes place in a single reaction tube or well and is complete in about three hours (Figure 1). No intermediate purification steps or sample transfers are necessary, thus preventing handling errors and loss of valuable samples.

Rapid Plus DNA Lib Prep Kit for Illumina® V2 includes the DNA Repair Enzymes II components. The DNA Repair Enzymes II components are designed to repair damaged DNA, mending the following damage types: nicks, gaps, oxidized bases, damaged/blocked 3 ends, AP sites (apurinic/apyrimidinic sites), and uracil bases.

Pairing the Rapid Plus DNA Lib Prep Kit for Illumina® V2 with the Truncated DNA Adapter Kit for Illumina® Set_C/Set_D adds the capability to multiplex up to 96 NGS-ready libraries. Once purified and quantified, the resulting libraries are ready for Illumina® NGS instruments using standard Illumina® sequencing reagents and protocols. The kit provides excellent results for high-coverage deep sequencing, such as de novo sequencing, whole genome resequencing, whole exome sequencing, and other enrichment techniques.

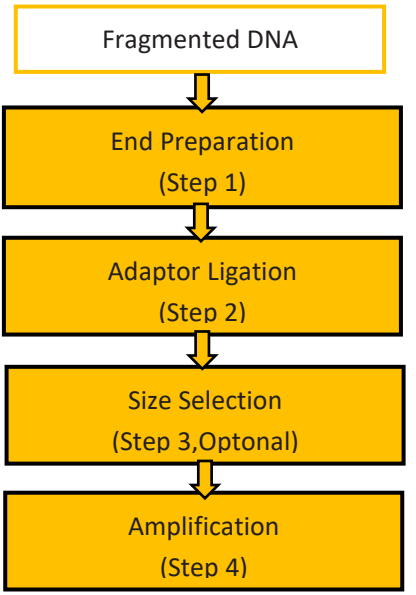

Figure 1. The following workflow illustrates the procedures involved in the Rapid Plus DNA Lib Prep Kit V2. The Rapid Plus DNA Lib Prep Kit for Illumina® V2 workflow consists of three simple steps that take place in the same PCR tube or well and eliminates the need to purify and transfer sample material.

2. List of Components

All components should be stored at -20°C. The shelf life of each reagent is one year when stored properly.

Table 1. Kit Contents

	Tube name	8 RXN	24 RXN	96 RXN
End Preparation	End Prep Buffer II	56 µl	168 µl	672 µl
	End Prep Enzymes	24 µl	72 µl	288 µl
	Damaged DNA Repair Enzymes II	24 µl	72 µl	288 µl
Adaptor Ligation	Ligation Buffer	240 µl	720 µl	2880 µl
	Ligation Enzymes II	80 µl	240 µl	960 µl
Amplification	Gloria Nova HS 2X PCR Mix for NGS	200 µl	600 µl	2400 µl
	Illumina®PCR Primer Mix	40 µl	120	480 µl

3. Additional Materials Required

- 100% ethanol (80% ethanol needs to be prepared for immediate use)
- Nuclease-free water
- PCR strips, tubes, or plates
- Magnetic stand
- Pipette tips with filters
- Thermocycler
- Microcentrifuge

- Vortex mixer
- Pipettes and multichannel pipettes
- Agilent Bioanalyzer, or comparable method to assess the quality of DNA library
- Magnetic Purification Beads：AgencourtTM AMPure XP bead (Beckman Coulter Inc., cat. no. A63880)
- Multiplex adapters (cat. # RK20292-21293, RK20294) compatible with Illumina® platforms

4. Precautions

Make sure to use high-quality DNA samples. Heavily nicked or damaged DNA will significantly lower library yield. We recommend Qubit® or other fluorometric methods to quantify DNA input. Residual trace RNA, nucleotides, ssDNA, or other contaminants may decrease the efficiency of the included library preparation enzymes.

5. Protocol

Step 1. End Preparation

1.1 Prepare end-preparation reaction mix in PCR tubes on ice according to Table 2 below.

Table 2. End-preparation Reaction Setup (per sample)

Component	Volume
Fragmented DNA	X µl
End Prep Buffer II*	7 µl
End Prep Enzymes	3 µl
Damaged DNA Repair Enzymes II**	3 µl
Nuclease-free Water	Up to 60 µl
Total volume	60 µl

* End Prep Buffer II color is yellow.

** For cfDNA or high-quality DNA samples, do not add Damaged DNA Repair Enzymes II (DDREs II). For FFPE or difficult samples, DDREs II is recommended; if not being used, the cycling protocol is as follows:
20°C /30min, 65°C /30min, 4°C /Hold.

1.2 Mix thoroughly by pipetting.

1.3 Incubate reactions in a thermocycler according to the program listed in Table 3. Set heated lid set temperature to 75°C.

Table 3. End-preparation Reaction Program

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

Step 2. Adaptor Ligation

2.1 Prepare and dilute adapters in low-EDTA TE buffer or nuclease-free water according to the instructions in Table 4 below.

Table 4. Adapter Dilution

Input DNA	Adaptor Dilution	Adapter Concentration
1 μg-10 ng	No dilution	15 μM
< 10 ng	2-fold	7.5 μM

2.2 Prepare the ligation reaction mix in PCR tubes on ice according to Table 5.

Note：The water, buffer and ligation enzymes should be premixed and then added in a single pipetting step. The working adaptor must be added separately.

Table 5. Ligation Reaction Setup

Component	Vomule
End Prep Reaction Mix (Step 1.3)	60 μl
Ligation Buffer	30 μl
ddH_2O	5 μl
Ligation Enzymes II	10 μl
Working Adaptor（**Table 4**）	5 μl

Total volume	110 µl

2.3 Incubate at 20°C for 15 minutes in a thermocycler WITHOUT a heated lid, and then hold at 4°C.

2.4 Clean up ligated DNA. Optional **Size Selection** can be performed during this procedure according to the protocol in Step 3 (page 8).

2.4.1 Add 88 µl (ratio 0.8X) of AgencourtTM AMPure XP bead to each sample and mix well by pipetting.

2.4.2 Incubate the mixture at room temperature (RT) for 5 minutes.

2.4.3 Pellet the beads on a magnetic stand at RT for 2 minutes. Carefully remove and discard the supernatant (avoid disturbing pelleted beads).

2.4.4 Wash the beads with 200 µl fresh 80% ethanol. Pellet the beads on a magnetic stand and carefully remove the ethanol.

2.4.5 Repeat step 2.4.4 for a total of two washes.

2.4.6 With the PCR tube on the magnetic stand, use a 10 µl pipette to remove remaining ethanol, then leave tube lid open to air dry the pellet for 2 minutes.

2.4.7 If proceeding to **amplification**, resuspend the magnetic beads in 21 µl nuclease-free water. For **size selection**, resuspend the magnetic beads in 51 µl nuclease-free water.

2.4.8 Mix thoroughly by pipetting, then incubate at RT for 1 minute to release the DNA from the beads. Spin down if necessary.

2.4.9 Pellet the beads on a magnetic stand at RT for 2 minutes.

2.4.10 Transfer 20 µl (or 50 µl) of the supernatant to a new PCR tube. Store the library at -20°C until ready for library quantification.

Safe stopping point: The purified joint ligation product can be temporarily stored at 4°C/-20°C for 1-2 weeks.

Step 3. Size Selection (optional)

3.1 Guide for size selection using magnetic beads.

Table 6. Ratios for using AgencourtTM AMPure XP bead for DNA Size Selection

Median Insert Size(bp)	150~350
Library Size (bp)	250~500
1st Binding Beads	0.7X (35 µl)
2nd Binding Beads	0.2X (10 µl)

3.2 Add 1st Binding Beads into 50 µl sample of adapter-ligated DNA (from step 2.4.10) according to the volume ratio described in Table 6 and mix thoroughly by pipetting.

3.3 Incubate at RT for 5 minutes.

3.4 Pellet the beads on a magnetic stand at RT for 2 minutes. Carefully transfer the supernatant to a new PCR tube (avoid disturbing pelleted beads).

3.5 Add 2nd Binding Beads to the supernatant according to the ratio in Table 6 and mix thoroughly by pipetting.

3.6 Incubate at RT for 5 minutes.

3.7 Pellet the beads on a magnetic stand at RT for 2 minutes. Carefully remove and discard the supernatant.

3.8 Wash the beads with 200 µl fresh 80% ethanol. Pellet the beads on a magnetic stand and carefully remove the ethanol.

3.9 Repeat Step 3.8 for a total of two washes.

3.10 Keeping the PCR tube on the magnetic stand, use a 10 µl pipette to remove remaining ethanol at the bottom of the tube. With tube lid open, air dry the pellet for 2 minutes.

3.11 Resuspend magnetic beads in 21 µl nuclease-free water.

3.12 Mix thoroughly by pipetting, then incubate at RT for 1 minute to release the DNA from the beads. Spin down if necessary.

3.13 Pellet the beads on a magnetic stand at RT for 2 minutes. Transfer 20 µl of the supernatant to a new PCR tube for amplification.

Safe stopping point: The purified joint ligation product can be temporarily stored at 4°C/-20°C for 1-2 weeks.

Step 4. Amplification

This kit accommodates either full-length or truncated adapters suitable for the Illumina® platform. Please select the library amplification system in Table 7, Table 8, and Table 9 according to adapter type.

4.1 Prepare the PCR reaction according to the Table 7 or 8.

Table 7. PCR Amplification Reaction Setup（full-length adapter）

(Adaptors：Cat.NO RK20292、RK20293)

Component	Volume
Adapter-Ligated DNA	20 μl
Gloria Nova HS 2X PCR Mix for NGS	25 μl
10 PCR Primer Mix	5 μl
Total volume	50 μl

Table 8. PCR Amplification Reaction Setup（truncated adapter with single index）

（Adaptors：Cat.NO RK20294、RK20295）

Component	Volume
Adapter-Ligated DNA	20 μl
Gloria Nova HS 2X PCR Mix for NGS	25 μl
Universal PCR Primer	2.5 μl
PCR Index	2.5 μl
Total volume	50 μl

4.2 Mix thoroughly by pipetting.

4.3 Program a thermocycler according to the Table 10 below with total amplification cycles according to Table 11.

Table 10. PCR Cycles for Library Amplification

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

Table 11. Number of PCR Amplification Cycles

Input DNA（ng）	Number of cycles to achieve a 1 μg library yield*
1000	2-4
500	4-5
250	5-6
100	6-7
50	7-8
25	8-10
10	10-12
1	14-16
0.1	18-20

*Note: 1. Add an additional 1-3 amplification cycles for FFPE samples.

2. Choose the higher number of cycles for PCR amplification when using Illumina® full-length adapters.

4.4 Add 50 µl (ratio 1.0X) of AgencourtTM AMPure XP bead to each reaction tube and mix thoroughly by pipetting.

4.5 Incubate at RT for 5 minutes.

4.6 Pellet the beads on a magnetic stand at RT for 2 minutes. Carefully remove and discard the supernatant (avoid disturbing pelleted beads).

4.7 Wash the beads with 200 µl fresh 80% ethanol. Pellet the beads on a magnetic stand and carefully remove the ethanol.

4.8 Repeat Step 4.7 for a total of two washes.

4.9 Keeping the PCR tube on the magnetic stand, use a 10 µl pipette to remove remaining ethanol at the bottom of the tube. With tube lid open, allow the pellet to air dry for 2 minutes.

4.10 Resuspend the magnetic beads in 31 µl of low-EDTA TE buffer. Mix thoroughly by pipetting and then incubate at RT for 1 minute to release the DNA from the beads.

4.11 Pellet the beads on a magnetic stand at RT for 2 minutes.

4.12 Transfer 20 µl of clear supernatant to a new PCR tube.

4.13 Store the library at -20°C until ready for library quantification or sequencing.

Safe stopping point: The purified joint ligation product can be temporarily stored at 4°C/-20°C for 1-2 weeks.

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