EpiNext™ CUT&LUNCH Library Preparation Kit

Base Catalog # P-1058

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiNext™ CUT&LUNCH Library Preparation Kit is a complete set of optimized reagents designed for, in a fast manner, highly sensitive CUT&LUNCH applications, including preparations of DNA library from low input samples to profile interactions between proteins and DNA via next generation sequencing using Illumina platforms.

Starting Materials: Enriched DNA from the CUT&LUNCH assay. The amount can be from 1 ng to 100 ng, with an optimized range of 10-50 ng.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the tube or vials. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

KIT CONTENTS

Component	24 Reactions Cat. #P-1058-24	2x 24 Reactions Cat. #P-1057-48	Storage Upon Receipt
10X Q-End Polishing Buffer*	60 µl	120 µl	-20°C
Q-End Polishing Enzyme Mix*	26 µl	52 µl	-20°C
2X Ligation Buffer*	500 µl	1000 µl	-20°C
T4 DNA Ligase*	26 µl	52 µl	-20°C
Adaptors (50 µM)*	30 µl	60 µl	-20°C
MQ Binding Beads	2x 2 ml	8 ml	4°C
2X HiFi PCR Master Mix*	320 µl	640 µl	-20°C
Universal Primer*	30 µl	60 µl	-20°C
Index Primer 1*	30 µl	60 µl	-20°C
Elution Buffer	2 ml	2x 2 ml	4°C

** Spin the solution down to the bottom prior to use.*

SHIPPING & STORAGE

The kit is shipped on frozen ice packs at 4°C.

Upon receipt, store the components according to the temperatures in the table above away from light.

All components of the kit are stable for 6 months from the date of shipment, when stored properly.

MATERIALS REQUIRED BUT NOT SUPPLIED

Equipment

- ☐ Vortex mixer
- ☐ Thermocycler with 48 or 96-well block
- ☐ Centrifuge including desktop centrifuge (up to 14,000 rpm)
- ☐ Magnetic device (96-well PCR plate format)
- ☐ Adjustable pipette and pipette tips
- ☐ 0.2 ml PCR vials

Reagents

- ☐ DNA sample
- ☐ 100% ethanol
- ☐ Distilled water

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the EpiNext™ CUT&LUNCH Library Preparation Kit is tested against predetermined specifications to ensure consistent product quality. EpigenTek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply call our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit, which can be accessed online at www.epigentek.com/datasheet.

Usage Limitation: The EpiNext™ CUT&LUNCH Library Preparation Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The EpiNext™ CUT&LUNCH Library Preparation Kit and methods of use contain proprietary technologies by EpigenTek.

A BRIEF OVERVIEW

DNA library preparation is a critical step for next generation sequencing (NGS). For generating accurate sequencing data in NGS, the prepared library DNA should be sufficient in yield and of high quality. Also, as NGS technology is continuously improving, DNA library preparation is required to be optimized accordingly. The amount of DNA enriched by CUT&LUNCH from low input cells or from low abundant target protein is often at low or sub-nanogram levels, which causes insufficient DNA library yields by using currently available library preparation methods. In addition, the currently used library preparation methods are time-consuming. To address this issue, EpigenTek developed the EpiNext™ CUT&LUNCH Library Preparation Kit, specifically suitable for use with the CUT&LUNCH assay. This kit has the following features:

- **High sensitivity and flexibility**: Can be used for both non-barcoded (singleplexed) and barcoded (multiplexed) DNA library preparation. The amount of input DNA can be as low as 1 ng with a range from 1 to 100 ng.
- **Extremely fast and streamlined procedure**: The procedure from enriched DNA to ready-to-use library is about only 1 hr. No clean-up is required between each step, and all reactions take place in the same tube before PCR, thereby saving time and preventing handling errors as well as loss of valuable samples.
- **The most convenient for use:** Contains all required components for each step of DNA library preparation, which are sufficient for end polishing, ligation, clean-up, size selection, and library amplification, thereby allowing the library preparation to be the most convenient with reliable and consistent results.

- **Minimized bias**: Ultra HiFi amplification enables researchers to reproducibly achieve high yields of DNA library with minimal sequence bias and low error rates.

PRINCIPLE & PROCEDURE

The EpiNext™ CUT&LUNCH Library Preparation Kit contains all reagents required at each step of the workflow for carrying out successful DNA library preparation with use of enriched DNA from the CUT&LUNCH assay. In the library preparation, the end repair/dA tailing (end polishing) of the enriched DNA is performed simultaneously. Adaptors are then ligated to both ends of the polished DNA fragments for amplification and sequencing. Ligated fragments are size selected and purified with MQ beads, which allows quick and precise size selection of DNA. Size-selected DNA fragments are amplified with a high-fidelity PCR mix that ensures maximum yields from minimum amounts of starting material and provides highly accurate amplification of library DNA with low error rates and minimum bias.

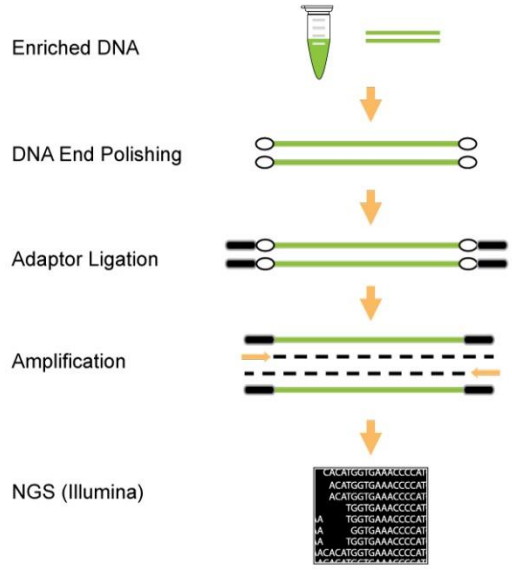

Fig 1. Schematic procedure of the EpiNext™ CUT&LUNCH Library Preparation Kit.

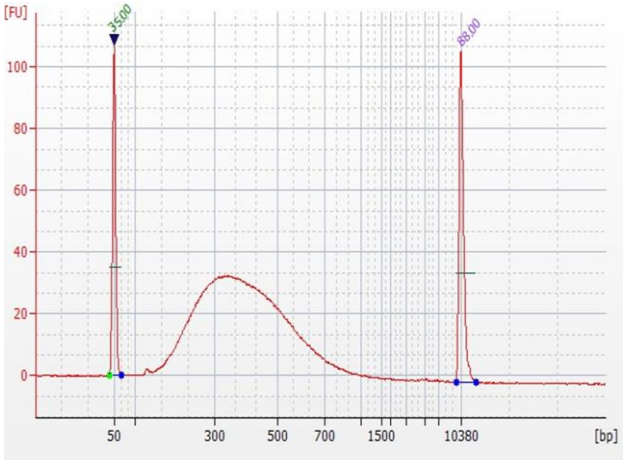

Fig 2. Size distribution of library fragments. Enriched DNA from the CUT&LUNCH assay was used for DNA library preparation by the EpiNext™ CUT&LUNCH Library Preparation Kit. The peak reflects the insert size of DNA fragments (170-180 bps).

Procedure overview and time table

Steps	Required time
Enriched DNA End Repair	10 min
Enriched DNA Ligation	20 min
Library Amplification and Purification	30 min

REACTION PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

1. End Repair Reaction

a. Prepare **End Repair Reaction Solution** required for each sample according to Table 1:

Table 1. End Repair Reaction

Component	Volume
Enriched DNA from CUT&LUNCH	10 µl
10X Q-End Polishing Buffer	1.5 µl
Q-End Polishing Enzyme Mix	1 µl
Distilled Water	2.5 µl
Total Volume	**15 µl**

b. Mix and incubate for 5 min at 20°C and 5 min at 72°C in a thermocycler (without heated lid).

Note: *The amount of enriched DNA can be 1-100 ng with an optimal amount of 10-50 ng.*

2. Target DNA Ligation

a. Prepare a reaction mix for adaptor ligation according to Table 2. Add the following reagents to a 0.2 ml PCR tube containing end polished DNA from Step 1b.

Table 2. Adaptor Ligation

Component	Volume
End polished DNA (from Step 1b)	15 µl
2X Ligation Buffer	17 µl
T4 DNA Ligase	1 µl
Adaptors	1 µl
Total Volume	**34 µl**

b. Mix and incubate for 10 min at 25°C in a thermocycler (without heated lid).

Note: *(1) The pre-annealed adaptors included in the kit are suitable for both non-barcoded (singleplexed) and barcoded (multiplexed) DNA library preparation and are fully compatible with Illumina platforms. (2) If using adaptors from other suppliers (both single-end and barcode adaptors), make sure they are compatible with Illumina platforms and add the correct amount (final concentration 1.5-2 µM, or according to the supplier's instruction).*

c. Resuspend **MQ Binding Beads** by vortexing. Add 40 µl of resuspended beads to the PCR tube of ligation reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.

d. Incubate for 4 minutes at room temperature to allow DNA to bind to the beads.

e. Put the PCR tube on an appropriate magnetic stand until the solution is clear (about 2 minutes). Carefully remove and discard the supernatant. *(**Caution: Be careful not to disturb or discard the beads that contain DNA.**)*

f. Keep the PCR tube in the magnetic stand and add 150 µl of freshly prepared *90% ethanol* to the tube, and then carefully remove and discard the ethanol.

g. Repeat Step 2f once for a total of two washes.

h. Resuspend the beads in 12 µl **Elution Buffer**, and incubate at room temperature for 4 minutes to release the DNA from the beads.

i. Capture the beads by placing the tube in the magnetic stand until the solution is completely clear.

j. Transfer 12 µl to a new 0.2 ml PCR tube for PCR amplification or store at -20C for later use.

Note: *Take 1 µl of eluted DNA and quantify the concentration of the ligated DNA by a fluorescence method (e.g., PicoGreen or Qubit) so that the library amplification cycles can be determined at step 3b of library amplification.*

3. Library Amplification

a. Prepare the PCR reactions.

Thaw all reaction components, including master mix, primer solutions, and DNA template. Mix well by vortexing briefly. Keep the components on ice while in use and return to -20˚C immediately following use. Add the components into the sample PCR tubes according to the following table:

Component	Size (µl)
2X HiFi PCR Master Mix	12.5 µl
Universal Primer	1 µl
Index Primer 1	1 µl
Adaptor Ligated DNA	10.5 µl
Total Volume	**25 µl**

Important Note: *Using **Index Primer 1** included in the kit will generate a singleplexed library. For multiplexed library preparation, replace **Index Primer 1** with one of the 12 or 24 different barcodes (indexes) contained in the **EpiNext™ NGS Barcode (Index) Set** (Cat. #P-1060, EpigenTek). You can also add user-defined barcodes (Illumina compatible) instead of **Index Primer 1**.*

b. Program the PCR reactions.

Place the reaction tubes in the instrument and set the PCR conditions as follows:

Cycle Step	Temp	Time	Cycle
Activation	98°C	30 sec	1
Cycling	98°C 55°C 72°C	10 sec 20 sec 20 sec	Variable*
Final Extension	72°C	2 min	1

** PCR cycles may vary depending on the ligated DNA concentration. In general, use 20 cycles for 8 ng, 23 cycles for 2 ng, and 28 cycles for 0.5 ng of the ligated DNA. Further optimization of PCR cycle number may be required.*

4. Clean-Up of Amplified Library DNA

a. Resuspend **MQ Binding Beads** by vortexing.

b. Add exactly 22.5 µl (0.9X) of the resuspended beads to the PCR tubes of the amplification reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.

c. Incubate for 4 min at room temperature to allow the DNA to bind to the beads.

d. Put the PCR tubes in the magnetic stand until the solution is clear (about 1 min). Carefully remove and discard the supernatant. (***Caution: Be careful not to disturb or discard the beads that contain the DNA.***)

e. Keep the PCR tubes in the magnetic stand and add 150 µl of freshly prepared *90% ethanol* to the tubes, then carefully remove and discard the ethanol.

f. Repeat Step 4e once for a total of two washes.

g. Resuspend the beads in 12 µl of **Elution Buffer**, and incubate at room temperature for 4 min to release the DNA from the beads.

h. Capture the beads by placing the tubes in the magnetic stand until the solution is completely clear.

i. Transfer 12 µl from each sample to a new 0.2 ml PCR tube.

Quality of the prepared libraries can be assessed using an Agilent Bioanalyzer® or other comparable methods. Library fragments should have the correct size distribution (e.g., 200-400 bps at peak size) without adaptors or adaptor dimers.

To check the size distribution, dilute the library 5-fold with water and apply it to an Agilent high sensitivity chip. If there is presence of <150 bp adaptor dimers or of larger fragments than expected, they should be removed. To remove fragments below 150 bps, use 0.8X **MQ Binding Beads** according to sub-steps a - i of Step 4 of “Clean-Up of Amplified Library DNA”.

Store the prepared library at -20ºC until ready to use for sequencing.

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
Low yield of library	Insufficient amount of starting DNA.	To obtain the best results, the amount of input DNA should be >10 ng.
	Insufficient purity of starting DNA.	Ensure that RNA is removed by RNase treatment before starting the library preparation protocol.
	Improper reaction conditions at each reaction step.	Check if the reagents are properly added and incubation temperature and time are correct at each reaction step, including End Polishing, Adaptor Ligation, Size Selection, and Amplification.
	Improper storage of the kit.	Ensure that the kit has not exceeded the expiration date. Standard shelf life, when stored properly, is 6 months from the date of shipment.
Unexpected peak size of Agilent Bioanalyzer® trace: Presence of <150 bp adaptor dimers or presence of larger fragments than expected.	Improper ratio of **MQ Binding Beads** to DNA volume in size selection.	Check if the correct volume **of MQ Binding Beads** is added to the DNA solution accordingly. Proper ratios should remove the fragments of unexpected peak size.
	Insufficient ligation.	Too much or too little input DNA may cause insufficient ligation, which can shift the peak size of the fragment population to be shorter or larger than expected. Make sure that the ligation reaction is properly processed with the proper amount of input DNA.
	Over-amplification of library.	PCR artifacts from over-amplification of the library may cause the fragment population to shift higher than expected. Make sure to use the proper PCR cycles to avoid this problem.

RELATED PRODUCTS

DNA Enrichment Reaction
P-2028 EpiNext™ CUT&RUN-Fast Kit
P-2033 EpiNext™ CUT&LUNCH-Seq Kit
P-2035 EpiNext™ CUT&LUNCH Assay Kit

Chromatin Accessibility Detection
P-1047 EpiQuik™ Chromatin Accessibility Assay Kit
P-1071 EpiNext™ Chromatin Accessibility Sequencing Fast Kit
P-1072 EpiNext™ In Situ Accessible Chromatin (ISAC) Preparation Kit
P-1073 EpiNext™ In Situ Accessible Chromatin Sequencing (ISAC-seq) Kit

Library Prep
P-1057 EpiNext™ ISAC Library Preparation Kit

NGS Barcode
P-1060 EpiNext™ NGS Barcode (Index) Set