EpiNext™ Chromatin Accessibility Sequencing Fast Kit

Base Catalog # P-1071

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiNext™ Chromatin Accessibility Sequencing Fast Kit is a complete set of optimized reagents designed for conducting a genome-wide analysis of chromatin accessibility, including nucleosome/transcription factor positioning from various biological samples via next generation sequencing.

Starting Materials: Starting materials can include various mammalian tissue or cell samples such as cells from flask or microplate cultured cells, fresh and frozen tissues, etc.

Input Amount of Cell/Tissues: The amount of cells/tissues for an optimal reaction can be from 50,000 cells or 5 mg tissues to 500,000 cells or 50 mg tissues.

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.

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KIT CONTENTS

Component	12 Reactions Cat. #P-1071-12	24 Reactions Cat. #P-1071-24	Storage Upon Receipt
CA Lysis Buffer	1 ml	2 ml	RT
CA Wash Buffer	8 ml	15 ml	4°C
CA Enzyme Mix*	65 µl	130 µl	-20°C
CA Cleavage Buffer	4 ml	8 ml	4°C
Cleavage Stop Buffer	0.5 ml	1 ml	RT
10X End Polishing Buffer*	30 µl	60 µl	-20°C
End Polishing Enzyme Mix*	13 µl	26 µl	-20°C
2X Ligation Buffer*	250 µl	500 µl	-20°C
T4 DNA Ligase*	15 µl	30 µl	-20°C
Adaptors (50 µM)*	15 µl	30 µl	-20°C
MQ Binding Beads*	2 ml	2 X 2 ml	4°C
MQ Binding Solution*	1 ml	2 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	0.5 ml	1 ml	RT

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

SHIPPING & STORAGE

The kit is shipped in two parts: the first part at ambient room temperature and the second part on frozen ice packs at 4°C.

Upon receipt: (1) Store **CA Enzyme Mix**, **10X End Polishing Buffer**, **End Polishing Enzyme Mix**, **2X Ligation Buffer**, **T4 DNA Ligase**, **Adaptors**, **2X HiFi PCR Master Mix 1**, **Universal Primer**, and **Index Primer** at –20°C away from light; (2) Store **CA Wash Buffer**, **CA Cleavage Buffer, MQ Binding Beads**, and **MQ Binding Solution** at 4°C away from light; (3) Store remaining components at room temperature away from light.

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

Note: *Check **CA Wash Buffer** and **Cleavage Stop Buffer** for salt precipitates before use. If present, briefly warm at room temperature or 37°C and shake the buffer until the salts are re-dissolved and clear.*

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Vortex mixer
- ☐ 1 ml Dounce homogenizer (for tissue samples)
- ☐ Scalpel or scissors (for tissue samples)
- ☐ 70 µm strainer mesh strainer (for tissue samples)
- ☐ 60 mm dish (for tissue samples)
- ☐ Centrifuge, including desktop centrifuge (up to 14,000 rpm)
- ☐ Thermal cycler
- ☐ Agilent® Bioanalyzer® or comparable method to assess the quality of the DNA library
- ☐ 96-well format magnetic stand
- ☐ Pipette and pipette tips
- ☐ 0.2 ml PCR tubes
- ☐ 1.5 ml microcentrifuge tubes
- ☐ 90% ethanol
- ☐ Distilled water
- ☐ Cells or tissues
- ☐ Trypan blue solution
- ☐ 1X PBS

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the EpiNext™ Chromatin Accessibility Sequencing Fast 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: EpiNext™ Chromatin Accessibility Sequencing Fast Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: EpiNext™ Chromatin Accessibility Sequencing Fast Kit and methods of use contain proprietary technologies by EpigenTek.

A BRIEF OVERVIEW

Determination of chromatin accessibility through mapping of the nucleosome positioning along the genome is directly linked to epigenetic gene regulation and chromatin states and structure. There are several methods currently used for detecting chromatin accessibility. The traditional method involves employing DNase I (DNase-seq) or micrococcal nuclease (MNase-seq) as tools to assess chromatin accessibility. However, these methods may have drawbacks that limit them from broad-spectrum use. They require a significant amount of starting material and are susceptible to assay bias due to challenges regulating enzyme concentrations and digestion duration. Moreover, they are unable to function in a harsh environment and are unsuitable for tissue samples.

The assay for transposase-accessible chromatin with next generation sequencing (ATAC-Seq) is a technique that was first described as an alternative advanced method for MNase-seq, FAIRE-seq, and DNase-seq. In ATAC-seq, intact nuclei are directly used, and accessible DNA regions are identified by probing open chromatin with hyperactive mutant Tn5 transposase that inserts sequencing adapters into open chromatin regions. This method is shown to be faster and more sensitive in epigenome analysis than DNase-seq or MNase-seq. However, ATAC-seq has significant fragmentation and GC content bias because of sequence-specific cleavage of Tn5 enzyme. Additionally, it is disadvantaged by the prevalence of mitochondrial contamination due to non-specific insertion of DNA into both mitochondrial DNA and nuclear DNA.

The EpiNext™ Chromatin Accessibility Sequencing Fast Kit developed by EpigenTek combines the advantages of both DNAase-seq/MNase-seq and ATAC-seq and reduces their drawbacks with the following features:

- **Extremely fast three-step protocol:** Completes the procedure (from cell/tissue sample to sequencing-ready library DNA) in just 1 hour and 55 minutes, faster than ATAC-seq. Minimizes nuclear damage, preserves chromatin structure, and avoids mitochondrial contamination.
- **Versatile usage**: Works with small cell amounts of fresh/frozen tissues, even in harsh environments, unlike DNAse-seq/MNase-seq.
- **Reduced biases:** Absence of sequence-specific cleavage minimizes fragmentation bias and biases related to GC or AT content.
- **Comprehensive kit:** Includes all necessary components for each step, ensuring convenience and cost-effectiveness of the EpiNext™ Chromatin Accessibility Sequencing Fast Kit

PRINCIPLE & PROCEDURE

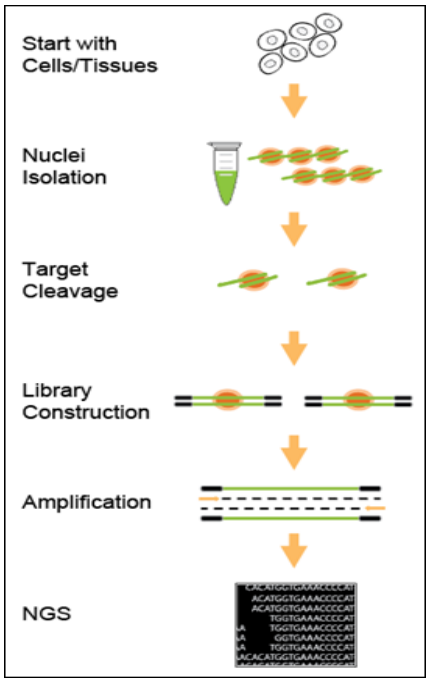

Fig 1. Schematic Procedure of the EpiNext™ Chromatin Accessibility Sequencing Fast Kit.

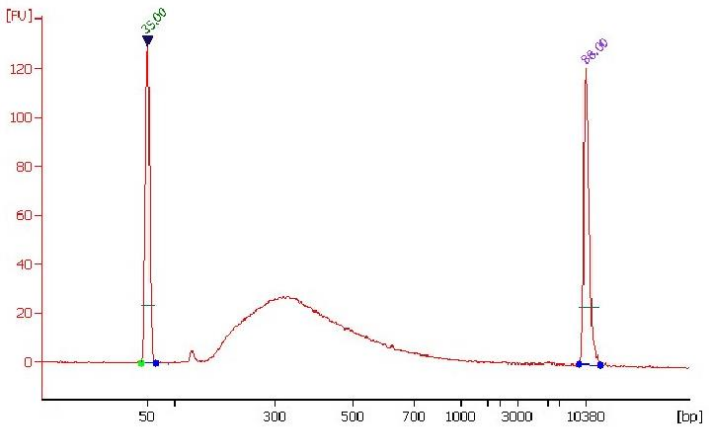

Fig 2. Characterization of EpiNext™ Chromatin Accessibility Sequencing DNA library. Chromatin isolated from 100,000 MCF-7 cells was cleaved and used for DNA library preparation with the EpiNext™ Chromatin Accessibility Sequencing Fast Kit. The library was analyzed with a Bioanalyzer®. The representative library

contains approximately 170 – 800 bp fragments with peak at around 320 bps as shown on the electropherogram.

The EpiNext™ Chromatin Accessibility Sequencing Fast Kit contains all the necessary reagents required for obtaining a genome-wide analysis of chromatin accessibility from cell/tissue samples via NGS. In this assay, nuclei are isolated from the cells/tissues and are exposed to a unique nucleic acid cleavage enzyme mix. Chromatin is fragmented, and DNA sequences at both ends of the target chromatin are cleaved/removed. At the same time, the DNA sequence occupied by the protein/histone is unaffected. The adaptors are ligated to the protein/histone-bound DNA fragments. The ligated DNA is then released, purified, and amplified with a high-fidelity PCR mix for library DNA construction.

Procedure overview and time table

Steps	Required time
Cell lysis, nuclei isolation, and cleavage	35 min
Adaptor ligation reaction	40 min
Index PCR amplification and clean-up	40 min

REACTION PROTOCOL

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

Starting Materials

Starting materials can include various mammalian tissue or cell samples such as cultured cells from a flask or microplate, fresh and frozen tissues, etc. Input amounts of cells/tissues for each reaction can be from 50,000 cells or 5 mg tissues to 500,000 cells or 50 mg tissues. For an optimal reaction, the input amount should be about 200,000 cells or 20 mg tissues.

1. Cell/Tissue Collection and Lysis

For Monolayer or Adherent Cells:

a. Grow cells (treated or untreated) to 80%-90% confluency on a plate or flask, then trypsinize and collect them into a 15 ml conical tube. Count the cells using a hemocytometer by trypan blue staining and aliquot 200,000 cells into a 1.5 ml microcentrifuge tube.

b. Centrifuge the cells at 500 x g (2500 rpm) for 5 min in a desktop centrifuge at room temperature. Discard the supernatant carefully.

c. Wash cells with 1 ml of ice-cold PBS once by centrifugation at 500 x g (2500 rpm) at room temperature for 5 min to pellet the cells. Discard the supernatant carefully.

d. Add **CA Lysis Buffer** (50 µl/1x10^5 cells) and pipet up and down 3 times gently to resuspend the cells and then incubate on ice for 5 min.

e. Add 500 µl of **CA Wash Buffer**, and invert the tube 3 times gently.

f. Centrifuge at 500 x g (2500 rpm) at room temperature for 10 min to pellet the nuclei.

For Suspension Cells:

a. Collect cells (treated or untreated) into a 15 ml conical tube. Count the cells using a hemocytometer by trypan blue staining and aliquot 200,000 cells into a 1.5 ml microcentrifuge tube.

b. Centrifuge the cells at 500 x g (2500 rpm in a desktop centrifuge) at room temperature for 5 min. Discard the supernatant.

c. Wash cells with 1 ml of ice-cold PBS once by centrifugation at 500 x g (2500 rpm) at room temperature for 5 min to pellet the cells. Discard the supernatant carefully.

d. Add **CA Lysis Buffer** (50 µl/1x10^5 cells) and pipet up and down 3 times gently to resuspend the cells and then incubate on ice for 5 min.

e. Add 500 µl of **CA Wash Buffer**, and invert the tube 3 times gently.

f. Centrifuge at 500 x g (2500 rpm) at room temperature for 10 min to pellet the nuclei.

For Tissues:

a. Put the tissue sample into a 60 mm dish. Remove unwanted tissue such as fat and necrotic material from the sample using a scalpel or scissors.

b. Weigh the sample (20 mg tissue) and cut the sample into small pieces (1-2 mm^3) with a scalpel or scissors.

c. Transfer the tissue pieces to a 1 ml Dounce homogenizer (5-50 mg tissue, with optimization of 20 mg is required for each reaction).

d. Add 200 µl of **CA Lysis Buffer**.

e. Disaggregate tissue pieces by 30 strokes.

f. Filter with a 70 µm mesh strainer and transfer homogenate to a 1.5 ml microcentrifuge tube. Count the cells using a hemocytometer by trypan blue staining. Invert the sample to gently mix and aliquot 200,000 cells into a 1.5 ml microcentrufuge tube.

g. Add **CA Lysis Buffer** (50 µl/1x10^5cells) and pipet up and down 3 times gently to resuspend the cells and then incubate on ice for 5 min.

h. Add 500 µl **of CA Wash Buffer**, and invert the tube 3 times gently

i. Centrifuge at 500 x g (2500 rpm in a desktop centrifuge) at room temperature for 10 min to pellet the nuclei.

2. Cleavage Reaction

a. During the centrifugation, prepare the cleavage reaction mix according to the following table. For each reaction, add the following components and mix:

Table 1. Cleavage Reaction Mix Preparation

Component	Volume (ul) per reaction
CA Cleavage Buffer	48
CA Enzyme Mix	2
Total	**50**

b. Immediately after centrifugation of the samples, carefully remove supernatant as much as possible and make sure that the nuclei pellet is not disturbed as the nuclei pellet is not visible and remaining supernatant may dilute the cleavage reaction mix.

c. Add 50 µl of the cleavage reaction mix to each nuclei sample. Gently resuspend the nuclei pellet by pipetting up and down 3-4 times.

d. Tightly cap the tubes and incubate at 37°C (waterbath or thermal cycler) for 2 min.

e. After 2 min reaction, add 10 µl of **Cleavage Stop Buffer** to each vial and incubate at room temperature for 1 min.

f. Resuspend **MQ Binding Beads** by vortexing. Add 120 µl (2X) of the resuspended beads to the samples. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.

g. Incubate for 4 min at room temperature to allow the DNA to bind to the beads and then transfer to a new 0.2 ml PCR tube.

h. Put the PCR tubes in the magnetic stand until the solution is clear (about 2 min). Carefully remove and discard the supernatant. *(Caution: Be careful not to disturb or discard the beads that contain the DNA.)*

3. DNA Ligation

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

Table 2. End Polishing Reaction Solution Preparation

Component	Volume
10X End Polishing Buffer	1.5 µl
End Polishing Enzyme Mix	1 µl
Distilled water	12.5 µl
Total Volume	**15 µl**

b. After removing the supernatant, (Step 2h), add 15 µl of **End Repair Reaction Solution** to each reaction tube. Mix and incubate for 10 min at 20°C in a thermocycler (without heated lid).

c. After the end polishing reaction, prepare a reaction mix for adaptor ligation by adding the ligation reagents to a 0.2 ml PCR tube containing end polished DNA (from Step 3b) according to Table 3.

Table 3. Adaptor Ligation Reaction

Component	Volume
End polished DNA (from step 3b)	15 µl
2X Ligation Buffer	17 µl
T4 DNA Ligase	1 µl
Adaptors	1 µl
Total volume	**34 µl**

d. Mix and incubate for 15 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, such as HiSeq™ sequencers. (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).*

4. Clean-up Ligated DNA

a Add 70 µl of **MQ Binding Solution** to the PCR tube of ligation reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.

b. Incubate for 5 minutes at room temperature to allow the DNA to bind to the beads.

c. 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.*

d. Keep the PCR tube in the magnetic stand and add 200 µl of freshly prepared *90% ethanol* to the tube. Incubate at room temperature for 1 min, and then carefully remove and discard the ethanol.

e. Repeat Step 4d once for total of two washes.

f. Resuspend the beads in 22 µl **Elution Buffer**, and incubate at room temperature for 5 minutes to release the DNA from the beads.

g. Capture the beads by placing the tube in the magnetic stand for 2 minutes or until the solution is completely clear.

h. Transfer 21 µl to a new 0.2 ml PCR tube for PCR amplification.

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 the step 5 of library amplification.*

5. Library Amplification

a. Prepare the PCR reactions.

Thaw all reaction components, including master mix, primer solution, 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 according to the following table:

Table 4. PCR Reaction Mix for Library Amplification

Component	Volume
2X HiFi PCR Master Mix	25 µl
Universal Primer	2.5 µl
Index Primer 1	2.5 µl
Ligated DNA	20 µl
Total Volume	**50 µl**

***Important Note*:** *Use of **Index Primer 1** included in the kit will generate a singleplexed library. For multiplexed library preparation, replace **Index Primer 1** with one of the12 different barcodes (indexes) contained in the **EpiNext™ Barcode (Index) Set-12** (#P-1060, EpigenTek).*

b. Program the PCR reactions.

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

Table 5. PCR Reaction Conditions for Library Amplification

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

** PCR cycles may vary depending on the PCR instruments and purified ligated DNA amount. In general, using 16 cycles are sufficient for library amplification. If not, further optimization of PCR cycle number may be required.*

6. Clean-Up of Amplified Library DNA

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

b. Add exactly 45 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 5 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 2 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 6e once for a total of two washes.

g. Resuspend the beads in 12 µl of **Elution Buffer**, and incubate at room temperature for 5 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 (about 1 min).

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. If there is presence of <150 bp adaptor dimers, they should be removed. To remove fragments below 150 bps, use 0.8X **MQ Binding Beads** according to sub-steps a - i of Step 5 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 ligated DNA.	Check ligated DNA concentration to obtain the best PCR conditions and results.
	Sample loss in the protocol, particularly at the step of nuclei pellet collection as the pellet is often not visible.	During centrifugation, place tubes in the microcentrifuge with hinges pointed towards the center of the rotor. This positioning ensures the location of the cell pellet, allowing easy removal of supernatant using a pipette from the side of the tube opposite the pellet.
	Improper PCR conditions during the amplification	Check if the reagents are properly added and PCR program and time are correct.
	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 date of receipt.
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.
	Over-cleavage of samples.	It may cause insufficient ligation, which can shift the peak size of the fragment population to be shorter than expected. Make sure that the cleavage reaction is properly processed with the proper amount of samples.

RELATED PRODUCTS

Chromatin Preparation

P-2001	ChromaFlash™ Chromatin Extraction Kit
P-2023	ChromaFlash™ Chromatin Isolation/Shearing Kit

DNA Isolation and Cleanup

P-1003	FitAmp™ General Tissue Section DNA Isolation Kit
P-1004	FitAmp™ Plasma/Serum DNA Isolation Kit
P-1006	DNA Concentrator Kit
P-1009	FitAmp™ Paraffin Tissue Section DNA Isolation Kit
P-1017	FitAmp™ Urine DNA Isolation Kit
P-1018	FitAmp™ Blood and Cultured Cell DNA Extraction Kit

DNA Enrichment Reaction

P-1015	Methylamp™ Methylated DNA Capture (MeDIP) Kit
P-1038	EpiQuik™ Hydroxymethylated DNA Immunoprecipitation (hMeDIP) Kit
P-1052	EpiQuik™ MeDIP Ultra Kit
P-2002	EpiQuik™ Chromatin Immunoprecipitation (ChIP) Kit
P-2003	EpiQuik™ Tissue Chromatin Immunoprecipitation Tissue (ChIP) Kit
P-2014	EpiQuik™ Plant ChIP Kit
P-2025	ChromaFlash™ One-Step ChIP Kit
P-2026	ChromaFlash™ One-Step Magnetic ChIP kit
P-2027	ChromaFlash™ ChIP Ultra Kit
P-2030	EpiNext™ ChIP-Seq High Sensitivity Kit
P-2028	EpiNext™ CUT&RUN Fast Kit

Chromatin accessibility Detection

P-1047	EpiQuik™ Chromatin Accessibility Assay Kit

PCR Analysis

P-1029	EpiQuik™ Quantitative PCR Kit

DNA Library Prep

P-1051	EpiNext™ DNA Library Preparation Kit (Illumina)
P-1053	EpiNext™ High Sensitive DNA Library Prep Kit (Illumina)

NGS Barcode

P-1060	EpiNext™ NGS Barcode (Index) Set-12

For ChIP-grade antibodies, search "chip-grade" at www.epigentek.com.

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