EpiNext™ CUT&LUNCH Assay Kit

Base Catalog # P-2035

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiNext™ CUT&LUNCH Assay Kit is a complete set of optimized reagents designed for, in a fast manner, enriching a protein (histone or strong binding transcription factor)-specific DNA complex directly from cells to profile interactions between proteins and DNA via qPCR or next generation sequencing using Illumina platforms.

Starting Materials: Starting materials can include various mammalian cell samples such as culture cells from a flask or plate, primary cells, or rare cell populations isolated from blood, body fluid, fresh/frozen tissues, and specific cells sorted from entire cell populations and embryonic cells, etc.

Input Amount of Cells: The amount of cells can be 2×10^3 to 5×10^5 cells per reaction. For optimal preparation, the cell input amount should be 2×10^5, although the results for modified histones can be obtained with as few as 500 cells.

Antibodies: Antibodies should be ChIP-grade in order to recognize the proteins that are bound to DNA or other proteins. If you are using antibodies that have not been validated for ChIP, then an appropriate control antibody, such as anti-RNA Polymerase II, anti-H3K4me3, or anti-H3K9me3 should be used to demonstrate that the antibodies are suitable for ChIP.

Internal Controls: Both negative and positive controls for CUT&LUNCH use are provided in this kit.

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	24 Reactions Cat# P-2035-24	Storage receipt
WB (Wash Buffer)	30 ml	4°C
PB (Permeabilization Buffer)	4 ml	RT
PIC (Protease Inhibitor Cocktail)*	30 µl	4°C
NDE (Nuclear Digestion Enhancer)	300 µl	RT
CEM (Cleavage Enzyme Mix)*	60 µl	-20°C
Positive Control Ab (H3K4me3,1 mg/ml)*	8 µl	-20°C
Non-Immune IgG (1 mg/ml)*	25 µl	4°C
CSS (Cleavage Stop Solution)	30 µl	RT
PDB (Protein Digestion Buffer)	5 ml	RT
Proteinase K (10 mg/ml)*	100 µl	4°C
Affinity Beads	100 µl	4°C
DPS (DNA Purification Solution)	600 µl	RT
DNA Binding Beads	60 µl	4°C
Elution Buffer	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, store the components away from light according to the temperatures in the table above.

Note: *Check if **WB (Wash Buffer)** and **PB (Permeabilization Buffer)** contain salt precipitates before use. If so, briefly warm at room temperature or 37°C and shake until the salts are re-dissolved.*

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
- ☐ Microscope and cell counter
- ☐ Thermocycler with 48 or 96-well block

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- ☐ Centrifuge including desktop centrifuge (up to 14,000 rpm)
- ☐ Rotator or rolling shaker
- ☐ Magnetic device (96-well PCR plate format)
- ☐ Adjustable pipette and pipette tips
- ☐ 0.2 ml PCR vials
- ☐ 1.5 ml microcentrifuge tubes

Reagents

- ☐ PBS
- ☐ Antibodies of interest
- ☐ Cell sample
- ☐ 100% ethanol
- ☐ 100% isopropanol
- ☐ Distilled water

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of EpiNext™ CUT&LUNCH Assay 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: A 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™ CUT&LUNCH Assay Kit is for research use only and is not intended for diagnostic or therapeutic application.

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

A BRIEF OVERVIEW

Enrichment of histone or transcription factor (TF)-complexed DNA in vivo, followed by qPCR and/or next-generation sequencing, offers an advantageous tool for studying genome-wide protein-DNA interactions. The major method commonly used to achieve this goal is chromatin immunoprecipitation

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followed by sequencing (ChIP-seq). This method includes highly specific ChIP-exo and ChIP-nexus. These two assays provide high-resolution mapping. However, the major limitation of these assays is that they need a large amount of input material, cells, or tissue to produce a strong enough signal over background noise. CUT&RUN (Cleavage Under Target & Release Under Nuclease) was developed for mapping protein-DNA interaction with limited biological materials, which requires much less sample amount and also significantly improved mapping resolution. However, a considerable drawback of this assay is non-specific cleavage by antibody un-coupled pAG-MNase, significantly limiting the specificity of the CUT&RUN use for most transcription factors in different species and cell/tissue types. In addition, the original CUT&RUN has complicated steps and needs to optimize assay conditions, which is still time-consuming.

To address these issues, EpigenTek has developed a new assay, the EpiNext™ CUT&LUNCH (Cleavage Under Target and Liberate Unique Nucleic Complex Homogenously) Assay Kit for studying in vivo DNA-protein interaction, which integrates all the advantages and overcomes the drawbacks of ChIP and CUT&RUN. The CUT&LUNCH assay has the following advantages and features:

- **Extremely Fast 3-Step Protocol:** Start directly from intact cells without needing ConA immobilization. The procedure can be completed in 1 hour and 50 minutes, minimizing nuclear damage and chromatin loss while preserving native chromatin structure.
- **Enhanced Specificity and Minimized Background:** Unique nucleic acid cleavage enzymes ensure low sequence bias by selectively cleaving DNA at both ends of the target protein/DNA complex, without affecting the DNA bound to the protein. Non-specific protein-DNA complexes are selectively eliminated, enabling high-resolution mapping of target protein-enriched regions.
- **Low Input Materials:** Robust unbound DNA cleavage and immunocapture are completed in minimal time. This method uses both cells and tissues while providing maximal degradation protection of the target protein and minimal sample loss. As a result, the input cell amount can be as few as 500 cells.
- **Wide Suitability:** Suitable for cultured cells and fresh or frozen tissues from various species, compatible with histones and transcription factors while maintaining assay specificity and sensitivity.
- **Highly Convenient:** The kit includes all necessary components, making the EpiNext™ CUT&LUNCH Assay Kit both convenient and cost-effective.
- **Seamless Integration with Existing Pipelines:** Enriched DNA for NGS can be analyzed with existing, time-tested ChIP-seq bioinformatics software and tools.

PRINCIPLE & PROCEDURE

The EpiNext™ CUT&LUNCH Assay Kit contains all the necessary reagents required for enriching a protein (histone or strong binding transcription factor)-specific DNA complex to profile interactions between proteins and DNA from various cell samples via qPCR or NGS. In this assay, cells are permeabilized and exposed to the ChIP-grade antibody of interest. With the use of a unique nucleic acid cleavage enzyme mix, DNA sequences at both ends of the target chromatin regions are cleaved/removed. The liberated non-specific protein-DNA complexes are eliminated, and only antibody-bound complexes will be selectively recovered. The DNA fragments in the captured protein/DNA complex are purified and can be directly used for gene-specific qPCR or DNA library construction to profile interactions between proteins and DNA.

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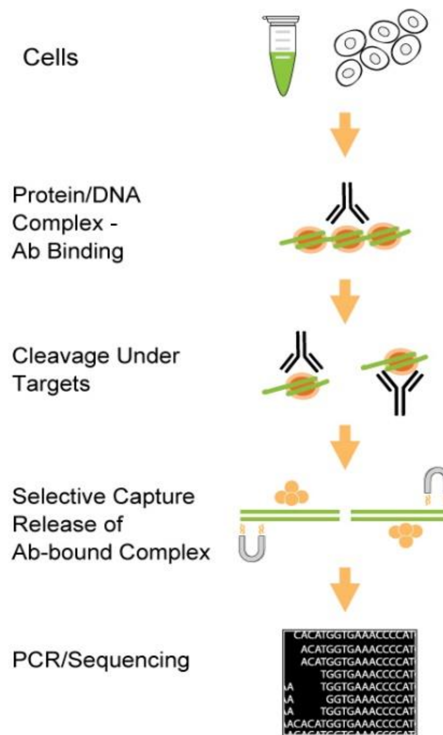

Fig 1. Schematic procedure of the EpiNext™ CUT&LUNCH Assay Kit.

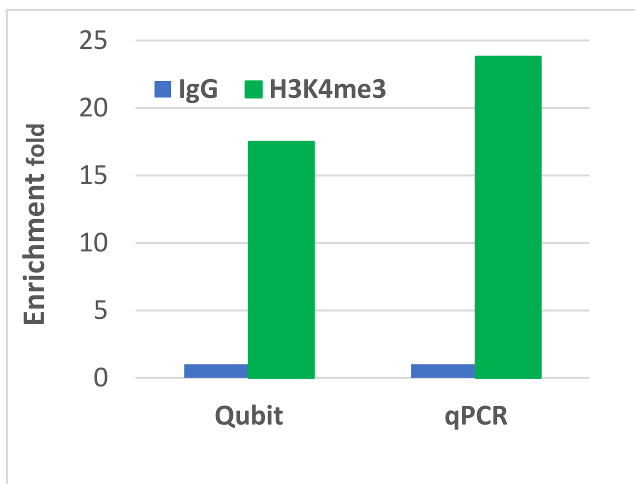

Fig 2. Target protein/DNA enrichment using the EpiNext™ CUT&LUNCH Assay Kit: Histone/DNA complex was captured by the positive control antibody (H3K4me3) from 200,000 MCF-7 cells. Non-immune IgG was used as the negative control. The enriched DNA was purified and fluorescently quantified by Qubit or analyzed by qPCR with targeting to the GAPDH promoter for enrichment fold comparison.

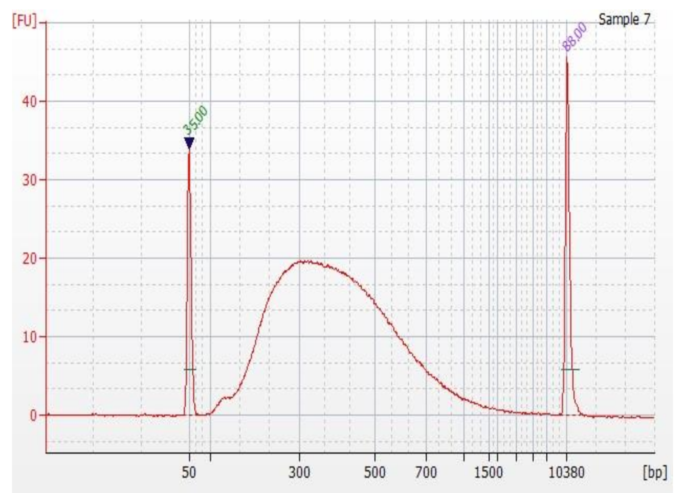

Fig 3. Size distribution of library fragments. Using the EpiNext™ CUT&LUNCH Assay Kit, histone-DNA complex was captured by the positive control antibody (H3K4me3) from 200,000 MCF-7 cells and used for DNA library preparation. The peak around 320 bps reflects the insert size of nucleosome DNA fragments (170 bps).

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Procedure overview and time table

Steps	Required time
Antibody binding to target	30 min
Enzyme cleavage	10 min
Selective recovery and purification	60 min

REACTION PROTOCOL

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

1. Preparation of Working Buffers

Prepare **Working PB buffer** by adding 1 µl of **PIC (Protease Inhibitor Cocktail)** to every 1 ml of **PB (Permeabilization Buffer)**.

2. Cell Collection and Antibody Capture

a. Isolate or collect the cells according to your own successful methods. These cells could include culture cells from a flask or plate, primary cells, or rare cell populations isolated from blood, body fluid, fresh/frozen tissues, specific cells sorted from entire cell populations and embryonic cells, etc. Count the cells using a hemocytometer by trypan blue staining. A total of 8-10 X 10^5 cells may be needed for each assay.

b. Wash cells with PBS by centrifugation at 2500 rpm for 5 min. Discard the supernatant.

c. Add 80-100 µl of **Working PB buffer** to re-suspend the cell pellet and count the cells using a hemocytometer by trypan blue staining. Adjust the cell concentration to be 1 X 10^4/µl if possible.

d. Prepare antibody-binding reaction by adding the reagents to 0.2 ml PCR tubes according to the following and mix well:

Reagents	Sample	Positive Control	Negative Control
Working PB buffer	76-78 µl	78 µl	78 µl
Your Antibodies	2-4 µl	0	0
Positive Control Ab	0	2 µl	0
Non-Immune IgG	0	0	2 µl
Total Volume	**80 µl**	**80 µl**	**80 µl**

Note: *The final amount of each component should be (a) antibodies of interest: 2 µg/tube; (b) positive control antibody: 2 µg/tube; and (c) non-immune IgG: 2 µg/tube.*

Shake the ***Affinity Beads*** *to completely suspend them before use.*

e. Transfer 20 µl of the suspended cells into each PCR tube, respectively as sample, positive control, and negative control. Mix and rotate on a rotator or rolling shaker at room temperature for 30 min.

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Meanwhile, transfer the remaining 20 µl of the suspended cells to a 0.2 µl PCR tube and use it as an "Input" (non-IP control).

The Input can be cleaved according to the following steps:

*Incubate at RT for 50 min and add 4 µl of **NDE (Nuclear Digestion Enhancer),** 1 µl of **CEM (Cleavage Enzyme Mix)** and incubate at room temperature for 2 min. Add 1 µl of **CSS (Cleavage Stop Solution)** and then spin down 10 sec in a mini centrifuge (6000-7000 rpm) to pellet cell debris. Transfer the supernatant to a new 0.2 ml PCR and then go to Step 4b.*

f. After the 30 min incubation, open the tube and add 4 µl of **NDE (Nuclear Digestion Enhancer)** and 1 µl of **CEM** (**Cleavage Enzyme Mix**), rotate, and incubate for an additional 10 min. Add 1 µl of **CSS (Cleavage Stop Solution)** and then spin down 10 sec in a mini centrifuge (6000-7000 rpm) to pellet cell debris. Transfer the supernatant to a new 0.2 ml PCR tube **(*Caution: Be careful not to disturb the pelleted cell debris). A***dd 4 µl of **Affinity Beads,** then rotate and incubate for an additional 20 min.

3. Cleavage Reaction

a. After the 20 min incubation, place the PCR tubes on the magnetic stand and wash each reaction tube three times with 150 µl of **WB (Wash Buffer)** and once with 150 µl of **PDB (Protein Digestion Buffer)** buffer. The wash with **WB (Wash Buffer)** can be performed as follows:

   After the solution has been removed, add **WB (Wash Buffer)** to the reaction tubes. Resuspend the beads by gently pipetting up and down several times. Ensure the pellets are completely resuspended and the beads do not cling to the pipette tips after pipetting. Place the tubes back in the magnetic stand for 1-2 min to pellet the beads. Then, remove and discard the solution from each reaction tube. Wash with **PDB (Protein Digestion Buffer)**, add the **PDB** to the tubes, then carefully remove and discard it.

4. DNA Purification

a. Prepare **Protein Digestion Solution** by mixing **Proteinase K** with **PDB (Protein Digestion Buffer)** at a 1:10 dilution [e.g., 1 µl of **Proteinase K** + 9 µl of **PDB (Protein Digestion Buffer)**].

b. Remove the tubes from the magnetic device after the last wash. Add 20 µl of **Protein Digestion Solution** to each reaction tube and "Input" tube. Mix and incubate at 55°C for 15 min in a thermocycler (without heated lid).

c. Put the tubes on the magnetic device until the solution is clear (about 2 min). Carefully transfer the solution from each sample to an unused PCR tube and mark it.

d. Add 20 µl of the **DPS (DNA Purification Solution)** into each tube containing the solution, followed by adding 40 µl of 100% isopropanol. Add 40 µl of the **DPS (DNA Purification Solution)** into the Input tube, followed by adding 80 µl of 100% isopropanol.

e. Resuspend **DNA Binding Beads** by vortexing. Add 2 µl of the resuspended beads to each tube. Mix thoroughly by pipetting up and down at least 10 times.

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f. Incubate for 5 min at room temperature to allow the DNA to bind to the beads.

g. Put the PCR tubes in the magnetic device 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).*

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

i. Repeat Step 4h once for a total of two washes.

j. Resuspend the beads in 15 µl of **Elution Buffer** and incubate at room temperature for 5 min to release the DNA from the beads.

k. Capture the beads by placing the tubes in the magnetic device until the solution is completely clear (about 1 min).

l. Transfer 15 µl from each sample to a new 0.2 ml PCR tube for immediate use or store at -20°C.

Note:

1) The DNA samples obtained can be directly used to construct DNA libraries for NGS analysis or used to perform a simple qPCR assay. We recommend using the EpiNext™ High-Sensitivity DNA Library Preparation Kit (Illumina) (#P-1053) for DNA library construction and the EpiQuik™ Quantitative PCR Fast Kit (#P-1029) for quantitative real time PCR analysis.

2) For the enrichment fold comparison of the positive control/negative control with qPCR, the primers specific for the positive target gene promoter region of H3K4me3, such as GAPDH, can be designed. For data analysis after real time PCR, fold enrichment (FE) can be calculated by simply using a ratio of amplification efficiency of the positive control DNA sample over that of the negative control sample with the appropriate primers.

$$FE = 2^{(NC\ CT - PC\ CT)} \times 100\%$$

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
Little or no enriched DNA from samples	Poor enrichment with antibody: some antibodies used in the reaction might not efficiently bind to the protein.	Increase the antibody amount and use validated ChIP-grade antibodies.
	Inappropriate cleavage condition.	The cleavage time may be too short or too long to cause the DNA fragment to be >1000 bps or <20 bps, respectively. Ensure the cleavage time and cleavage enzyme amount are correct.

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	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.
No difference in signal intensity between negative and positive control wells	Insufficient washing.	Check if washing recommendations at each step are performed according to the protocol. If the signal intensity in the negative control is still high, washing stringency can be increased in the following ways: 1. Increase wash time at each wash step: after adding **WB** (**Wash Buffer**), leave it in the wells for 2 min and then remove it. 2. Add one additional wash with **WB** (**Wash Buffer**), respectively: the provided volume of **WB** (**Wash Buffer**) is sufficient for 4 extra washes for each sample.
	For PCR verification, too many PCR cycles were used: plateau phase of amplification caused by excessive number of PCR cycles in endpoint PCR may mask the difference of signal intensity between negative control and positive control.	Decrease the number of PCR cycles (i.e., 32-35 cycles) to keep amplification at the exponential phase. This will reduce high background in endpoint PCR and allow differences in amplification to be seen. Real time PCR is another choice in such cases.
Poor results in downstream qPCR	Little or no PCR product even in the positive control.	Ensure that all PCR components were added and that a suitable PCR program is used (PCR cycle should be >35).
		PCR primers and probes were not appropriate or were incorrectly designed. Ensure the primer and probes are suitable for amplification of the DNA region of interest and the target regions to be amplified are less than 120 bps.
		Ensure the amount of template DNA used in PCR was sufficient.
	Significant non-specific PCR products.	Failed chromatin digestion. Ensure that all steps of the digestion and cleanup protocol were followed and that input DNA amount is within the recommended range.
		Primers and probes are not specific for target genes. Check the primer and probe design.

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RELATED PRODUCTS

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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