EpiNext™ CUT&RUN Fast Kit

Base Catalog # P-2028

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiNext™ CUT&RUN Fast Kit is designed to quickly enrich specific DNA complexed with a protein (histone or transcription factor) from low input cells/chromatin and to identify or map in vivo protein-DNA interaction by next generation sequencing using Illumina platforms or other methods such as qPCR. The innovative working principle, optimized protocol, and components of the kit allow for the capture of targeted protein/DNA complexes with minimized non-specific background levels. The captured DNA is specifically suitable for the construction of both non-barcoded (singleplexed) and barcoded (multiplexed) libraries to map target protein-DNA interaction regions with less bias and at a high resolution.

Input Amount: For cells**,** in general, the amount can be 2 x 10^3 to 2 x 10^5 cells per reaction. For optimal preparation, the cell input amount should be 1 x 10^5, although sequencing data obtained from the EpiNext™ CUT&RUN Fast Kit for modified histones can be obtained with as few as 500 cells. For chromatin isolated from cells or tissues, the amount can be 0.1 µg to 5 µg of chromatin per reaction. For optimal preparation, the chromatin input amount should be 2 µg.

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 (pre-prepared chromatin), and specific cells sorted from entire cell populations and embryonic cells, etc.

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-H3K27me3 should be used to demonstrate that the antibodies are suitable for ChIP.

Internal Controls: Both negative and positive ChIP controls are provided in this kit.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the PCR tubes. 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-2028-24	Storage Upon Receipt
WB (Wash Buffer)	30 ml	4°C
LB (Lysis Buffer)	4 ml	RT
CB (Capture Buffer)	4 ml	RT
NDE (Nuclear Digestion Enhancer)	300 µl	RT
PIC (Protease Inhibitor Cocktail)*	30 µl	4°C
CEM (Cleavage Enzyme Mix)*	60 µl	-20°C
Control Antibody (H3K9me3,1 mg/ml)*	8 µl	-20°C
Non-Immune IgG (1 mg/ml)*	25 µl	4°C
Control Chromatin (100 ng/µl)*	20 µl	-20°C
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	1000 µl	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 according to the temperatures in the table above away from light. The kit can be stable for up to 6-months from the date of shipment when stored properly.

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

MATERIALS REQUIRED BUT NOT SUPPLIED

Equipment

- ☐ Vortex mixer
- ☐ Thermocycler with 48 or 96-well block
- ☐ Centrifuge including desktop centrifuge (up to 14,000 rpm)
- ☐ Agilent® Bioanalyzer® or comparable method to assess the quality of DNA library
- ☐ Microplate 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

- ☐ Antibodies of interest
- ☐ Cell sample or chromatin
- ☐ 100% ethanol
- ☐ Distilled water

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the EpiNext™ CUT&RUN 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 contact 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&RUN Fast Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The EpiNext™ CUT&RUN Fast 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 next-generation sequencing offers an advantageous tool for studying genome-wide protein-DNA interactions. It allows for the analysis of a specific protein binding to DNA sequences in living cells. Such analysis requires that the method reliably identify true target protein-enriched regions, particularly from limited cell samples. These samples could include rare cell populations isolated from tissues, specific cells sorted from entire cell populations, and primary cultured sub-population cells such as embryonic cells. In addition, the method should ensure that enriched DNA contains minimal background and that the mapping of protein-DNA binding regions is with minimal bias and at high resolution. A major method that has been used for a long time to achieve this goal is chromatin immunoprecipitation, followed by sequencing (ChIP-Seq). However, the main limitation for ChIP is that it needs 1) a large amount of

input material, cells or tissue, to produce a strong enough signal over background noise; and 2) cross-linking during an initial fixation step**.** There are several advanced methods available for ChIP-Seq with reduced cell numbers or increased resolution. These methods include ChIP-exo and ChIPmentation. ChIP-exo provides high-resolution mapping, but is time-consuming and requires an ample supply of input cells. While ChIPmentation uses transposase and sequencing-compatible adaptors to enable the integration of ligation during the ChIP process, it follows a traditionally slow (2 days) ChIP procedure and cannot achieve high-resolution mapping. CUT&RUN (Cleavage Under Target & Release Using Nuclease) was developed to release the captured target protein/DNA complex from limited biological materials for mapping protein-DNA interaction, and it has significantly improved mapping resolution. However, CUT&RUN needs expensive PA/Mnase fusion protein that has significant A/T sequence bias, causing the target protein-interacted DNA region profiles to be seriously affected by the level of MNase digestion. Therefore, as an improvement, we have developed a new technique: cleavage under target and recover using nuclease fast (CUT&RUN Fast) for enriching histone or TF-bound DNA. Our innovative approach combines the advantages of ChIP-exo and CUT&RUN with the fastest procedures and incorporates it into the EpiNext™ CUT&RUN Fast Kit. This kit has the following features:

- **High Enrichment:** Uses a unique nucleic acid cleavage enzyme mix, which has low sequence bias, to simultaneously fragment chromatin and cleave/remove any DNA sequences in both ends of the target protein/DNA complex without affecting DNA occupied by the target protein. The enriched DNA >20 bps can be efficiently and quickly recovered. Thus, the target protein-enriched regions can be reliably achieved and identified at high-resolution mapping.
- **Low Input Materials:** Robust sonication-free fragmentation, unbound DNA cleavage, and immunocapture are all processed in the same single-tube with on-beads ligation. This method uses both cells and tissues and allows for maximal degradation protection of the target protein with minimal sample loss. As a result, the input cell amount can be as few as 500 cells, or chromatin amount can be as low as 0.1 µg.
- **Minimal Background**: Cleavage of unbound DNA sequences in the two (2) end of the target protein/DNA complex in situ enables the minimized immunocapture background, allowing the sequencing data analysis with <10 million reads.
- **Fast, Streamlined Procedure**: The procedure from cells to library DNA is less than 3 hours.
- **Highly Convenient**: The kit contains all required components for each step of the CUT&RUN Fast, thereby allowing the EpiNext™ CUT&RUN Fast Kit to be the most convenient with reliable and consistent results.

PRINCIPLE & PROCEDURE

The EpiNext™ CUT&RUN Fast Kit contains all necessary reagents required for carrying out a successful CUT&RUN Fast starting from mammalian cells or isolated nuclei/chromatin. In the reaction, nuclei are isolated from cells. The target protein-DNA complex is bound/captured with the CHIP-grade antibody of interest. With the use of a unique nucleic acid cleavage enzyme mix, chromatin is fragmented, and DNA sequences in both ends of the target protein/DNA complex are cleaved/removed. At the same time, the DNA sequence occupied by the target protein is unaffected. The target protein-bound DNA is then purified and eluted. The enriched DNA can be used for the analysis of protein-DNA interaction with various methods, especially with next generation sequencing. Included in the kit are a positive control antibody (anti-H3K9me3), a negative control non-immune IgG, and control chromatin, which can be used to demonstrate the efficacy of the kit and performance at the PCR/bioanalyzer step.

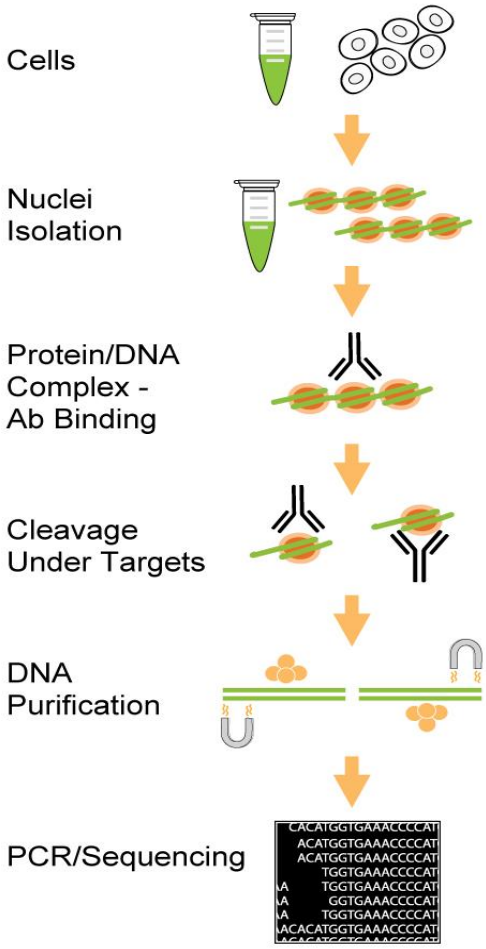

Fig 1. Workflow of the EpiNext™ CUT&RUN Fast Kit.

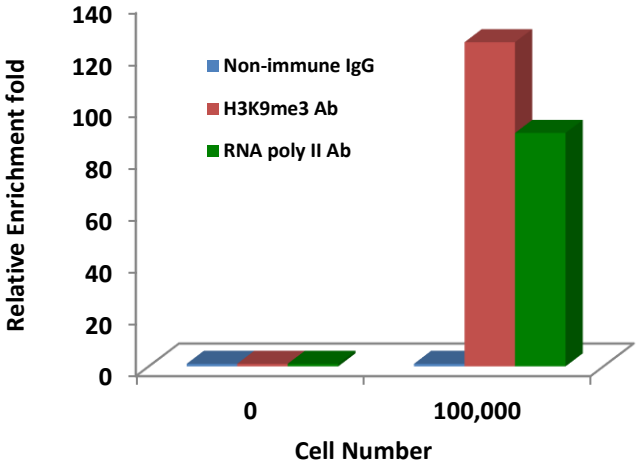

Fig 2. Target protein/DNA enrichment using the EpiNext™ CUT&RUN Fast Kit: Histone/DNA complex was captured by the control antibodies (anti-H3K9me3 and anti-RNA polymerase II, EpigenTek) from 100,000 Jurkat cells. Non-immuno IgG was used as control. The enriched DNA was purified and fluorescently quantified for enrichment fold comparison.

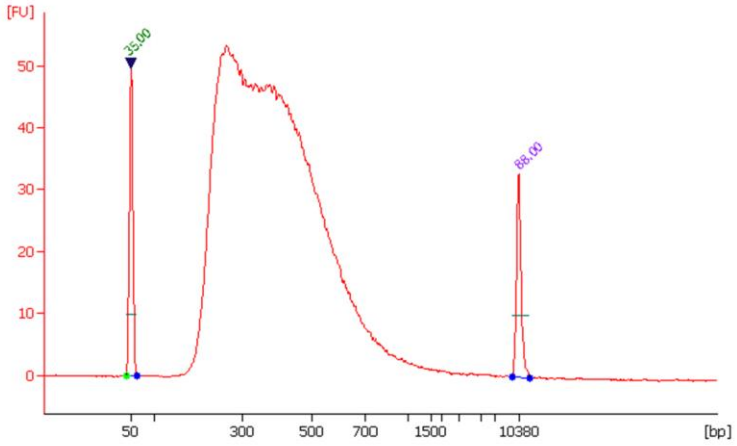

Fig 3. Size distribution of library fragments. Using the EpiNext™ CUT&RUN Fast Kit, histone-DNA complex was captured by the control antibody (H3K9me3) from 1 ug of chromatin isolated from Hela cells and used for DNA library preparation. Peak around 290 bps reflects the insert size of mononucleosome (150 bps).

Procedure overview and time table

Steps	Required time
Cell lysis and nuclei/chromatin isolation	20 min
Antibody capture and DNA cleavage	100 min
DNA release and recovery	40 min

ASSAY PROTOCOL

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

1. Preparation of Working Buffers

a. Prepare **Working Lysis Buffer** by adding 1 µl of **PIC** (**Protease Inhibitor Cocktail)** to every 1 ml of **LB (Lysis Buffer)**

b. Prepare **Working CB (Capture Buffer)** by adding 1 µl of **PIC** (**Protease Inhibitor Cocktail)** to every 1 ml of **CB (Capture Buffer).**

2. Cell Lysis, Immunocapture and Cleavage

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, and fresh/frozen tissues, specific cells sorted from entire cell populations and embryonic cells, etc.

Note:
1) If using tissues, the chromatin should be isolated and quantified. EpigenTek provides ***ChromaFlash™ Chromatin Extraction Kit (Cat# P-2001).*** *The same volume (100 µl) of isolated chromatin can be added for the reaction at Step 2d.*

2) In general, the negative control paired with each sample may be needed for qPCR analysis or optionally needed for sequencing library construction QC. Thus, the cells or chromatin for negative control use should also be prepared accordingly.

b. Wash cells with PBS by centrifugation at 1000 rpm for 5 min. Discard the supernatant.

c. Add 110 µl of **Working Lysis Buffer** to re-suspend the cell pellet, and incubate at 4°C for 10 min. Vortex vigorously for 10 sec per 5 min. Meanwhile, prepare antibody-affinity beads solution by adding the reagents to 0.2 ml PCR tubes according to the following and mix well:

Reagents	Sample tube	Positive Control tube	Negative Control Tube
CB (**Capture Buffer**)*	92-94 µl	94 µl	94 µl
Your Antibodies	2-4 µl	0	0
Control Antibody	0	2 µl	0
Non-Immune IgG	0	0	2 µl
Affinity Beads	4 µl	4 µl	4 µl
Total Volume	**100 µl**	**100 µl**	**100 µl**

**This is not the Working Buffer*

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.*

d. Centrifuge the samples at 12,000 rpm (desk-top centrifuge) at 4°C for 10 min. Remove the supernatant and add 110 µl of **Working CB (Capture Buffer)** into the sample (nuclei pellet). Resuspend the nuclei pellet and transfer 100 µl of the suspended nuclei into the sample PCR tube (If starting cells are less than 1X 10^4, directly transfer 100 µl of cell suspension into the sample tube without centrifugation after incubation). For the positive control tube, add 100 µl diluted **Control Chromatin** solution (95 µl of **Working CB (Capture Buffer)** + 5 µl of **Control Chromatin.** For the negative control tube, also add 100 µl of the suspended nuclei or 100 µl the diluted **Control Chromatin** solution. Mix and rotate on a rotator or rolling shaker at room temperature for 90 min. Meanwhile, transfer the remaining 10 µl of the suspended nuclei to a 0.2 µl PCR tube and use it as an input.

<u>The input can be cleaved according to the following steps:</u>

1. Add 20 µl of **Working CB (Capture Buffer)** followed by adding 1.5 µl of **NDE (Nuclear Digestion Enhancer),** 1 µl of **CEM** (**Cleavage Enzyme Mix**) and incubate at room temperature for 5 min.
2. Add 3 µl of **Proteinase K** into the tube and incubate at 60°C for 15 min in a thermocycler (without heated lid). Then go to Step 3c for DNA release/recovery.

e After the 90 min incubation, open the tube and add 10 µl of **NDE (Nuclear Digestion Enhancer),** and 2 µl of **CEM (Cleavage Enzyme Mix),** then rotate and incubate for additional 10 min.

f. Put the tubes on 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).*

g Keep the PCR tubes in 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 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 are not clinging 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.

3. **Enriched DNA Release/Recovery**

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 sample, negative control, and positive control. Mix and incubate at 60°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.

d. Add 20 µl of the **DPS (DNA Purification Solution)** into each sample, negative control, and positive control tube followed by adding 160 µl of 100% Ethanol. Add 25 µl of the **DPS (DNA Purification Solution)** into Input tube followed by adding 200 µl of 100% Ethanol.

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.

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 3h once for a total of two washes.

j. Resuspend the beads in 20 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 20 µl from each sample to a new 0.2 ml PCR tube for immediate use or store at -20C.

Note:
1) The concentration of the enriched DNA can be simply quantified by a fluorescence method to know the enrichment fold with comparison between the positive control and the negative control or between the sample and the negative control. We recommend using EpigenTek's ***FitAmp General DNA Quantification Kit (#P-1020)*** *for enriched DNA quantification.*

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 H3K9me3 such as MyoD, Sat2a and ZNF554 can be designed. We recommend using EpigenTek's ***EpiQuik Quantitative PCR Fast Kit (#P-1029)*** *for the qPCR assay.*

3) For use of the enriched DNA to construct library for sequencing, we recommend EpigenTek's ***EpiNext High-Sensitivity DNA Library Preparation Kit (Illumina) (#P-1053)****.*

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
Little or no enriched DNA from both sample and positive control wells	Insufficient amount of cells.	Ensure the cell number is correctly counted. If the cell number is less than 1 x 10^4, whole cell suspension (without centrifugation) should be directly used for the immuno-cleavage reaction.
	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.
	Incorrect temperature and/or insufficient time during DNA release.	Ensure the incubation times and temperatures described in the protocol are followed correctly.
	Improper PCR conditions, including improper PCR programming, PCR reaction solutions, and/or primers when PCR is used for enriched DNA quantification.	Ensure the PCR is properly programmed. If using a homebrew PCR reaction solution, check if each component is correctly mixed. If using a PCR commercial kit, check if it is suitable for your PCR. Confirm species specificity of primers. Primers should be designed to cover a short sequence region (70-150 bp) for more efficient and precise amplification of target DNA region (the binding site of the protein of interest).
No difference in signal intensity between negative and positive control wells	Insufficient washing.	Check if washing recommendations at each step is 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 3-4 min and then remove it. 2. Add an additional one 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.

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 Quantification

P-1012 FitAmp™ Circulating DNA Quantification Kit
P-1020 FitAmp™ General DNA Quantification 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 (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 (Illumina)

PCR Analysis

P-1029 EpiQuik™ Quantitative PCR Kit

DNA Library Prep

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

NGS Barcode

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

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com