Hyperactive pG-MNase CUT&RUN Assay Kit for Illumina

NB-54-0341-01

Contents

Hyperactive pG-MNase CUT&RUN Assay Kit for Illumina

#Cat: NB-54-0341-01 Size: 24rxns

1/Product Description

Hyperactive pG-MNase CUT&RUN Assay Kit for Illumina is a protein-DNA interaction assay kit specially developed for Illumina high-throughput sequencing platforms. Cleavage Under Targets and Release Using Nuclease (CUT&RUN) is a new method for studying protein-DNA interactions. By fusing Protein G with transposase, under the guidance of the antibody, it allows accurate targeting of the target protein and cutting of the DNA sequence near the target site. With an optimized reaction system and library preparation workflow, this kit offers multiple advantages over conventional chromatin immunoprecipitation followed by sequencing (ChIP-seq), including high success rate, excellent antibody compatibility, short turnaround time, and ease of use, making It is especially suitable for such fields as early embryonic development, stem cells, cancer, and epigenetics. All reagents in this kit have undergone strict quality control and function testing to ensure the optimal and repeatability of library preparation.

2/Components

	Components	NB-54-0341-01 (24 rxns)
BOX 1	ConA Beads Pro	260 µl
BOX 2	FastPure gDNA Mini Columns	24
	Collection Tubes 2 ml	24
	Buffer GDP	24 ml
	Buffer GW	4 ml
BOX 3	pG-MNase Enzyme	30 µl
	MNase Dilution Buffer	2 × 1.3 ml
	5% Digitonin	1.3 ml
	10 × Binding Buffer	800 µl
	10 × Wash Buffer	12 ml
	Antibody Buffer(-)	2 × 1.2 ml
	$CaCl_2$	48 µl
	2 × Stop Buffer(-)	2 × 1.2 ml
	Spike in DNA (5 ng/µl)	24 µl
	DNA Damage Repair Enzyme	48 µl
	End Prep Buffer	240 µl
	End Prep Enzyme	120 µl
	Rapid Ligation Buffer	600 µl
	Rapid DNA Ligase	120 µl
	VAHTS HiFi Amplification Mix	600 µl
	PCR Primer Mix3 for Illumina	120 µl

▲ The color marked in the components table represents the color of tube cap of each component; FastPure gDNA Mini Columns: DNA absorption columns;
Collection Tubes 2 ml: Collect tubes for filtrate; Buffer GDP: DNA binding buffer;
Buffer GW: Washing buffer. Add absolute ethanol by the indicate volume on the bottle before use

3/Storage

BOX 1: Store ConA Beads Pro at 2 ~ 8°C and adjust the transportation method according to the destination
BOX 2: Store at 15 ~ 25°C and transport at room temperature
BOX 3: Store 5% Digitonin at -30 ~ -15°C; it can be stored at room temperature (15 ~ 25°C) for one week;
Store the other components at -30 ~ -15°C;
Transport at ≤ 0°C

4/Applications

This product is intended for studying protein-DNA interactions in mammalian cells with 5,000 - 500,000 input cells. Yeast, plant tissues and cells should be treated before proceeding with the assay using this kit.

5/Self-prepared Materials

Reagents

Antibodies: Primary antibody and secondary antibody (optional).
Protease inhibitor: Roche Complete Protease Inhibitor EDTA-Free Tablets is recommended.
Magnetic beads for purification: VAHTS DNA Clean Beads (Neo Biotech # NB-54-0060).
DNA adapters: VAHTS DNA Adapters for Illumina (Neo Biotech # NB-54-0066/ NB-54-0067/ NB-54-0070/ NB-54-0071/ NB-54-0072/ NB-54-0073).
NB-54-0066/ NB-54-0067 each provides 12 kinds of single-end 6 bp indexed adapters (24 kinds in total). # NB-54-0070/ NB-54-0071/ NB-54-0072/N8 NB-54-0073 each provides 24 kinds of single-end 8 bp indexed adapters (96 kinds in total).
Use an appropriate adapter kit according to the sample quantity. Others: Ethanol absolute and ddH2O

Instruments and Consumables

Rotary mixer;
Magnetic stand;
PCR thermal cycler;
Low-adsorption EP tubes, PCR tubes;
DNA quality control: Agilent Technologies 2100 Bioanalyzer or equivalents

6/Notes

For research only. Not for use in diaognostic procedures.
Due to many factors such as the samples, abundance of the target protein in the sample, and operation, it may be necessary to adjust the experiment process as per the actual situation. To obtain high-quality sequencing libraries, please read the following precautions carefully.

Contact Neo Biotech Technical Support for assistance in case of any issues during use: info@neo-biotech.com

6-1/About Magnetic Beads

◇The experiment uses two kinds of magnetic beads. The kit contains ConA Beads Pro intended for cell binding. VAHTS DNA Clean Beads (Neo Biotech # NB-54-0060) is recommended for library purification. Correctly distinguish the different magnetic beads before use.

◇General precautions for use of magnetic beads:

▲ Store the beads at 2 ~ 8°C. Do not freeze at -30 ~ -15°C.

▲ The magnetic beads should be equilibrated to room temperature (leave for 30 min at room temperature) before use and should always be used at room temperature.

▲ The magnetic beads should be thoroughly mixed by pipetting up and down every time before they are pipetted.

◇Precautions for use of ConA Beads Pro:

▲ Do not mix by shaking or pipetting vigorously after ConA Beads Pro bind to cells to avoid the release of cells from the beads.

▲Avoid prolonged exposure of the beads or bead-cell complexes to air to prevent adverse effects on subsequent tests due to the drying of beads.

▲Avoid high-speed centrifugation or prolonged attraction of magnetic beads on the magnetic separation rack to prevent bead aggregation.

▲ It is normal that some beads may adhere to the tube wall or aggregate during incubation. As long as the bead-cell complexes are immersed in the solution, the results of subsequent tests will not be affected. The degree of adhesion or aggregation varies across different types of cells that are bound to the beads. For the same type of cells, a larger sample input amount causes more pronounced bead aggregation or adhesion to the tube wall. If beads adhere to the tube wall or aggregate, gently flick the bottom of the tube to mix the bead-cell complexes. Avoid repeated uncapping or mixing by pipetting up and down.

◇Precautions for use of magnetic beads for DNA purification:

▲ After the sample and magnetic beads are thoroughly mixed and placed on the magnetic magnetic stand, pipette the supernatant only when the solution becomes completely clear and always leave 2 - 3 µl of supernatant. Avoid disturbing the magnetic beads during pipetting; otherwise, the yield may decrease. In this case, the magnetic beads can be mixed and placed on the magnetic stand for separation again. Given the varying performance of the magnetic stand, a longer separation time may be needed to separate the magnetic beads from the liquid completely.

▲Magnetic beads should be rinsed using 80% ethanol that is freshly prepared and equilibrated to room temperature. During rinsing, always keep the EP tube on the magnetic stand and do not disturb the magnetic beads.

▲ Air-dry the magnetic beads at room temperature before elution (the surface changes from glossy brown to matte brown). Insufficient drying may leave residual ethanol absolute and thereby affect subsequent tests, while over-drying may result in surface cracks and thereby reduce the purification yield. In general, the magnetic beads can be fully dried through air-drying at room temperature for 5 - 10 min. Do not dry by heating

6-2/About Sample Preparation and Antibody Selection

◇If live cells are used, common suspension cells can be harvested for the assay by centrifugation and removal of medium. For most adherent cells, digest with trypsin, collect the cell suspension, centrifuge, and discard the medium to collect cells. For some cell lines, trypsin digestion may affect the binding of cells to ConA Beads Pro, and the judgment can be performed according to the actual condition.

◇Cells used in the CUN&RUN experiment can be stained with trypan blue for cell viability assay. The cell viability is preferably >90%. Handle cells as gently as possible during the experiment to maintain cell viability. For cells with poor growth or dead cells, the binding state of protein and DNA will change, and even the protein may detach and become naked DNA. Random nuclease cleavage produces high background noise, severely affecting the experimental results.

◇For low-abundance target proteins in the sample and certain special transcription factors, light cross-linking of cells may help yield better experimental results.

◇It is recommended to incorporate positive and negative controls in the experiment. We recommend using a high-abundance histone, such as H3K4me3, as the positive control, and IgG as the negative control to identify any potential abnormality throughout the assay. It is not necessary to add non-specific IgG as a negative control as it does not provide valuable information in sequencing analysis. You may choose to include it as needed.

◇We recommend using chromatin immunoprecipitation (ChIP)-grade primary antibodies and IgG for the assay. If there are no commercially available ChIP-grade antibodies against the target protein, you may try using antibodies that are suitable for immune fluorescence (IF) assays.

◇For some special target proteins, you can choose to add the step of secondary antibody incubation. We recommend selecting an unmodified secondary antibody with high affinity to Protein G based on its source species.

6-3/About Column-based Extraction Reagents

◇Before the first use, add the designated volume of ethanol absolute to Buffer GW as indicated on the label of the reagent bottle and mark accordingly. Store at room temperature.

◇Buffer GDP precipitates when stored at low temperature. Let it stand at room temperature for a while before use, or prewarm it in a 37°C water bath until the precipitate is completely dissolved if necessary, and mix well before use.

◇Perform the extraction procedure at room temperature (15 ~ 25°C).

6-4/About Spike in DNA

◇The Spike in DNA supplied in this kit is a 300 bp fragment derived from E. coli λDNA. It can be used to normalize experimental data acquired under different treatments or cell conditions in quantitative analysis of test results.

◇Spike in DNA can be diluted with ddH2O. Dilute low-concentration Spike in DNA right before use and avoid long-term storage at low concentrations. We recommend determining the concentration before dilution and diluting based on the actual concentration to ensure accurate addition of Spike in DNA.

◇The Spike in DNA sequence is as follows. The sequence information is also available on our website (www.neo-biotech.com).

ATAACTCAATGTTGGCCTGTATAGCTTCAGTGATTGCGATTCGCCTGTCTCTGCCTAAT
CCAAACTCTTTACCCGTCCTTGGGTCCCTGTAGCAGTAATATCCATTGTTTCTTATATAA
AGGTTAGGGGGTAAATCCCGGCGCTCATGACTTCGCCTTCTTCCCATTTCTGATCCTC
TTCAAAAGGCCACCTGTTACTGGTCGATTTAAGTCAACCTTTACCGCTGATTCGTGGAA
CAGATACTCTCTTCCATCCTTAACCGGAGGTGGGAATATCCTGCATTCCCGAACCCATC GACGA

6-5/About Adapter Amount for Library Preparation

◇Neo Biotech provides two sets of indexed adapters for the Illumina sequencing platform, which can be selected according to the application scenario and the sample quantity. # NB-54-0066/ NB-54-0244-02 each provides 12 kinds of adapters with a single 6 bp index (24 kinds in total); # NB-54-0070/ NB-54-0071/ NB-54-0072/ NB-54-0073 each provides 24 kinds of adapters with a single 8 bp index (96 kinds in total).

◇The quality and input amount of adapters directly affect the preparation efficiency and library quality. Increasing the input amount of adapters can improve library yields to some extent. Excessive input of adapters may lead to residual adapters; insufficient input may affect the ligation efficiency and result in reduced library yield.

◇Adapters are diluted with ddH2O and can be stored at -30 ~ -15°C after dilution. It is recommended to dilute adapters right before use and avoid repeated freeze-thaw cycles.

◇Adapters should be diluted with ddH2O before being added to the reaction system. The adapters are recommended to be diluted at a ratio between 1:10 - 1:50 when the cell input amount is 10,000 - 500,000 or between 1:50 - 1:100 when the cell input amount is less than 10,000

6-6/About Cell Input Amount and Amplification Cycles

◇The kit is compatible with a cell input amount of 5,000 - 500,000. The minimum compatible cell input amount varies depending on cell types, antibody types, and abundance of the target protein in the sample. For initial experiments, 10,000 - 100,000 cells are recommended to investigate the relationship between the cell input amount and the number of amplification cycles in the CUT&RUN assay of the target protein.

Take H3K4me3, a histone with medium- to high-abundance in K562 cells, as an example. The relationship between the cell input amount, number of amplification cycles, and library yield is shown in the following table:

Number of Input Cells	Number of Cycles	Library Yield (Qubit assay)
5,000	17 - 20	5 - 30 ng/μl
10,000	13 - 16	
100,000	12 - 14	10 - 60 ng/μl
500,000	10 - 12	

◇Low cell input is not recommended for standard CUT&RUN assays. If a small amount of cells is input, the experiment process will need to be optimized to yield satisfying results.

◇The PCR amplification yield merely needs to be sufficient for sequencing. There is no need to conduct additional amplification cycles for a higher library yield. An excessive number of cycles may lead to various adverse effects such as over-amplification, increased amplification bias, increased duplications, increased chimeric products, and cumulative amplification mutations.

6-7/About Library Quality Control

In general, a prepared library can be evaluated through concentration and size distribution analyses.

◇Library concentration determination:

▲ There are two common methods for library concentration determination: one uses fluorescent dyes that bind to double-stranded DNA (dsDNA), such as Equalbit 1× dsDNA HS Assay Kit (Neo Biotech # NB-54-0027) and PicoGreen-based kits, and the other is quantitative PCR (qPCR)-based absolute quantification, such as VAHTS Library Quantification Kit for Illumina (Neo Biotech # NB-54-0110 - NB-54-0115).

▲Too many amplification cycles can cause non-specific annealing of melted amplification products, resulting in cross-annealing of non-complementary strands. These products migrate slowly in electrophoresis-based assays and diffuse in the high molecular weight region. They are made up of single-stranded sequences with correct sizes, which, after denaturation, can normally bind to the flow cell and be sequenced and thus have no significant impact on library sequencing. However, an over-amplified library contains many incomplete double-stranded structures, and when it is quantified with a fluorescent dye for dsDNA identification, the quantification result will be lower than the actual value. In contrast, qPCR-based library quantification has denaturation steps and is not affected by over-amplification.

6-8/Additional Precautions

◇Thaw all kit components at appropriate temperatures and mix each component thoroughly before use.

◇Store different buffers and reagents at appropriate conditions to avoid inactivation.

◇To avoid cross-contamination of samples, it is recommended to use filter pipette tips and raplace the pipette tip for different samples.

◇If handled improperly, PCR products are highly susceptible to aerosol contamination, which affects the accuracy of experimental results. Therefore, it is recommended to physically separate the PCR reaction system preparation area from the PCR product purification and detection areas, use dedicated equipment (e.g., pipettes), and regularly clean each laboratory area with RNase, RNA and DNA Remover (Neo Biotech # NB-54-0405) to keep the laboratory environment clean.

7/Experiment Process

7-1/Buffer Preparation

▲ The following procedure applies to one sample. Scale the volumes based on the actual number of samples to be tested.

1.Binding Buffer: Dilute 30 µl of 10 × Binding Buffer to 300 µl with 270 µl of ddH2O and mix well.

2.Wash Buffer: Add 100 µl of 50 × Protease Inhibitor to 500 µl of 10 × Wash Buffer. Add 4.4 ml of ddH2O and mix well.

▲ 50 × Protease Inhibitor: Dissolve one Protease Inhibitor tablet in 1 ml of ddH2O and store at - 30 ~ -15°C.

▲ The prepared Wash Buffer can be stored at 4°C overnight.

3.Antibody Buffer: Add 1 µl of 5% Digitonin to 100 µl of Antibody Buffer (-), mix well, and pre-cool on ice.

4.Dig-wash Buffer: Add 38 µl of 5% Digitonin to 3.8 ml of the Wash Buffer prepared in Step 2 and mix well.

▲ Digitonin is toxic. Ensure proper personal protection when preparing the solution. Prepare the buffer right before use because it cannot be stored for a long time after Digitonin is added.

5.Stop Buffer: Add 1 µl of 5% Digitonin to 100 µl of 2 × Stop Buffer (-). Add the appropriate amount of Spike in DNA according to the cell input amount, and pre-cool on ice.

▲ Spike in DNA can be diluted with ddH2O. It is recommended to dilute the DNA just before use and store at -30 ~ -15°C.

▲ Spike in DNA can be used for the normalization of experimental data. For profiling of H3K4me3 in K562 cells, the recommended Spike in DNA input amounts are listed in the following table:

Number of Input Cells	Spike in DNA Input Amount
5,000 - 10,000	1 pg
100,000	10 pg
500,000	50 pg

6.Before the first use, add the designated amount of ethanol absolute to Buffer GW as indicated on the label of the reagent bottle, and mark accordingly.

	Volume of Ethanol Absolute (ml)
	HD102-01
Buffer GW	20

7-2/ConA Beads Pro Treatment

1.Add 100 µl of Binding Buffer to a 1.5 ml EP tube.

2.Resuspend ConA Beads Pro thoroughly by gentle pipetting. Add 10 µl of ConA Beads Pro to the Binding Buffer in Step 1, mix gently, and place the mixture on the magnetic stand. After the solution becomes clear (about 2 min), discard the supernatant.

3.Remove the EP tube from the magnetic stand, add 100 µl of Binding Buffer, then gently pipette to fully mix (do not mix by vortexing).

4.Place the EP tube on the magnetic stand. After the solution becomes clear (about 2 min), discard the supernatant, and add 10 µl of Binding Buffer to resuspend the beads.

7-3/Cell Collection

▲ Perform all steps before cell permeabilization at room temperature to minimize the stress on cells. Avoid vigorous vortexing in the procedure.

1.Collect and count cells at room temperature.

2.Transfer the required number of cells to a 1.5 ml EP tube. Centrifuge at 2,500 rpm (600 × g) for 5 min at room temperature, and discard the supernatant.

3.Resuspend the cells in 500 µl of Wash Buffer at room temperature. Centrifuge at 2,500 rpm (600 × g) for 5 min, and discard the supernatant.

4.Add 100 µl of Wash Buffer to each sample to resuspend the cells.

7-4/Cell and ConA Beads Pro Incubation

1.Transfer 100 µl of cells to the EP tube containing treated ConA Beads Pro. After shaking and mixing, incubate at room temperature for 10 min, and shake 2 - 3 times during this period.

▲Do not mix by pipetting or vortexing after the cells are added.

2.Briefly centrifuge (<100 × g) to collect the reaction mix. Place the EP tube on the magnetic stand. After the solution becomes clear (about 2 min), discard the supernatant.

▲Do not centrifuge for too long, as this may cause the beads to aggregate at the bottom of the tube.

7-5/Primary Antibody Incubation

1.Add 100 µl of pre-cooled Antibody Buffer to each sample to resuspend the cell-magnetic bead.

2.Add the antibody to the EP tube according to the recommended immune concentration in the antibody manual, and invert to mix.

3.Collect the reaction solution at the bottom of the tube with short-spin centrifugation (Do not aggregate the magnetic beads at the bottom of the tube by prolonged centrifugal time), then place the EP tube at 2 ~ 8°C overnight.

▲We recommend including a positive control in the experiment.

7-6/pG-MNase Enzyme Incubation

1.Add 1 µl of pG-MNase Enzyme to 100 µl of MNase Dilution Buffer. Then add 1 µl of the diluted enzyme to 100 µl of Dig-wash Buffer to prepare the pG-MNase Enzyme premix. Mix by inversion and place on ice.

▲ Dilute pG-MNase Enzyme just before use to ensure optimal cleavage activity. To ensure the stability ofpG-MNase Enzyme, we recommend aliquoting the components at first use and storing it strictly at-30 ~ -15°C.

2.Briefly centrifuge the EP tube from 07-5/Primary Antibody Incubation to collect the reaction solution. Place the EP tube on the magnetic stand until the solution becomes clear (30 sec - 2 min), and discard the supernatant.

3.Add 800 µl of Dig-wash Buffer to the EP tube and invert several times to ensure that the buffer and cell-bead complex are thoroughly mixed.

4.Briefly centrifuge to collect the reaction solution. Place the EP tube on the magnetic stand. After the solution becomes clear (30 sec - 2 min), discard the supernatant.

5.Repeat Steps 3 - 4 (twice in total).

6.Add 100 µl of the pG-MNase Enzyme premix from Step 1 and invert several times to ensure that the buffer and cell-magnetic bead complexes are thoroughly mixed.

7.Incubate with rotation at 4°C for 1 h.

7.7/Fragmentation

1.Add 2 µl of CaCl2 to 98 µl of Dig-wash Buffer. Mix by inversion and place on ice.

2.Briefly centrifuge the EP tube from **7-6/pG-MNase Enzyme Incubation** to collect the reaction mix. Place the EP tube on the magnetic separation rack. After the solution becomes clear (30 sec - 2 min), discard the supernatant.

3.Add 800 µl of Dig-wash Buffer to the EP tube and invert several times to ensure that the buffer and cell-bead complex are thoroughly mixed.

4.Briefly centrifuge to collect the reaction mix. Place the EP tube on the magnetic separation rack. After the solution becomes clear (30 sec - 2 min), discard the supernatant.

5.Repeat Steps 3 - 4 (twice in total).

6.Add 100 µl of the CaCl2 premix from Step 1 and invert several times to ensure that the buffer and cell-bead complex are thoroughly mixed.
7.Immediately place the EP tube on ice and incubate for 60 - 90 min. Invert to mix for 2 - 3 times during this period.
▲Depending on the abundance of the target protein, the optimal MNase cleavage conditions are different, and should be adjusted accordingly. We recommend 60-min digestion on ice for common histones such as H3K4me3, 90-min digestion on ice for transcription factors such as CTCF, and longer digestion times as appropriate for low-abundance target proteins.

7-8/Fragmentation Termination and DNA Fragment Release
1.Add 100 µl of Stop Buffer to the EP tube from 07-7/Fragmentation, and mix well by inversion.
2.Incubate the EP tube in a 37°C water bath for 10 - 30 min.
▲Do not shake the tube in the meantime. Keep the tube still to release the DNA fragments.

7-9/DNA Extraction
1.Add 1 ml of Buffer GDP to the 1.5 ml EP tube from 07-8/Fragmentation Termination and DNA Fragment Release of DNA Fragments, mix well by vortexing, and incubate at room temperature for 10 min with mixing 2 - 3 times by inversion.
2.Briefly centrifuge to collect the droplets from the tube wall. Place a FastPure gDNA Mini Columns in a Collection Tube 2 ml. Transfer 650 µl of the sample solution to the spin column, and centrifuge at 12,000 rpm (13,400 × g) for 60 sec.
3.Discard the flow-through and place the spin tube in the Collection Tube. Transfer the rest of the sample solution to the spin tube, and centrifuge at 12,000 rpm (13,400 × g) for 60 sec.
4.Discard the flow-through and place the spin tube in the Collection Tube. Add 700 µl of Buffer GW (supplemented with ethanol absolute) to the spin tube. Centrifuge at 12,000 rpm (13,400 × g) for 60 sec.
▲ Add Buffer GW along the wall of the spin column, or cap the column and invert 2 - 3 times after adding Buffer GW to fully rinse the residual liquid on the column wall.
5.Discard the flow-through and place the spin tube in the Collection Tube. Centrifuge at 12,000 rpm (13,400 × g) for 2 min.
6.Leave the spin column uncapped at room temperature for 2 - 5 min.
▲Air dry the adsorptive membrane thoroughly to ensure DNA purity.
7.Place the spin tube in a new 1.5 ml EP tube. Add 22 µl of ddH2O to the center of the column, and incubate for 2 min.
8.Centrifuge at 12,000 rpm (13,400 × g) for 2 min. Discard the spin tube and store the extracted product at -30 ~ -15°C.
▲The product from Step 8 contains the target gene fragments, which can be stored at -30 ~ -15°C for 7 days.

7-10/Library Preparation
Step 1: DNA Damage Repair & End Preparation
This step is conducted to repair damaged target DNA.
1.Thaw End Prep Buffer, mix well by inversion, and prepare the following reaction system in a sterile PCR tube:

Components	Volume
DNA fragments	15 - 20 µl
DNA Damage Repair Enzyme	2 µl
End Prep Enzyme	5 µl
End Prep Buffer	10 µl
ddH_2O	To 65 µl

▲ Mix the prepared reaction system thoroughly by gently pipetting instead of vortexing, and centrifuge it briefly to collect the reaction solution to the bottom of the tube.

2.Place the PCR tube into the PCR instrument and perform the following reactions:

Temperature	Time
Heated lid 105°C	On
30°C	20 min
65°C	15 min
4°C	Hold

Step 2: Adapter Ligation

This step is for ligating adapters to the ends of the product from DNA Damage Repair & End Preparation.

1.Dilute adapters to the appropriate concentration based on the cell input amount and **6-5/About Adapter Amount for Library Preparation.**

2.Thaw Rapid Ligation Buffer and mix well by inversion before placing it on ice for later use. Prepare the following reaction system in a sterile PCR tube:

Components	Volume
End Preparation Product	65 µl
Rapid Ligation Buffer	25 µl
Rapid DNA Ligase	5 µl
DNA Adapter X	5 µl
In total	100 µl

▲ Mix the prepared reaction system thoroughly by gently pipetting instead of vortexing, and centrifuge it briefly to collect the reaction solution to the bottom of the tube.

3.Place the PCR tube into the PCR instrument and perform the following reactions:

Temperature	Time
Heated lid 105°C	On
20°C	15 min
4°C	Hold

4.Purify the reaction products using VAHTS DNA Clean Beads (Neo Biotech # NB-54-0060):

a.Mix VAHTS DNA Clean Beads thoroughly by vortexing after equilibrating the magnetic beads to room temperature.
b.Pipette 60 µl of VAHTS DNA Clean Beads into 100 µl of the adapter ligation products. Mix the entire reaction system thoroughly by vortexing or gently pipetting up and down 10 times. Incubate at room temperature for 5 min.
c.Centrifuge the PCR tube briefly and place it on a magnetic stand to separate the magnetic beads from the solution. Wait until the solution becomes clear (about 5 min), and carefully remove the supernatant without disturbing the magnetic beads.
d.Keep the PCR tube on the magnetic stand. Add 200 µl of freshly prepared 80% ethanol to rinse the magnetic beads. Incubate at room temperature for 30 sec, and carefully remove the supernatant.
e.Repeat Step d for a second rinse.
f.Keep the PCR tube on the magnetic stand. Uncap the tube and air-dry the magnetic beads for 3 - 5 min.
g.After the beads are dried, remove the PCR tube from the magnetic stand and elute with 22 µl of ddH2O. Mix the magnetic beads thoroughly by vortexing or gently pipetting up and down 10 times, and incubate at room temperature for 5 min.
h.Centrifuge the PCR tube briefly and place it on the magnetic stand to separate the magnetic beads from the solution. Wait until the solution becomes clear (about 5 min), and carefully pipette 20 µl of the supernatant into a new EP tube. Store at -30 ~ -15°C.
▲ The adapter ligation products from Step h can be stored at -30 ~ -15°C for 7 days. Avoid repeated freeze-thaw cycles.

2.Place the PCR tube into the PCR instrument and perform the following reactions:

Temperature		Time		cycles
95°C		3 min		
98°C 60°C	}	10 sec 5 sec	}	10 - 20
72°C		1 min		
4°C		Hold		

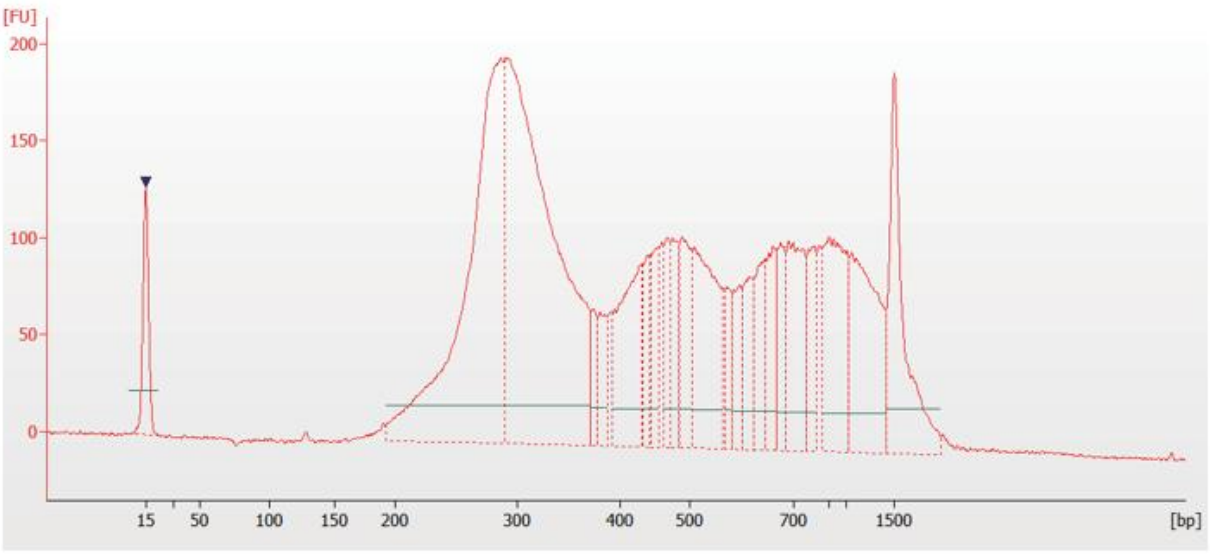
▲Select an appropriate number of amplification cycles based on the expression of the target protein.
3.Purify the reaction products using VAHTS DNA Clean Beads (Neo Biotech # NB-54-0060):
a. Mix VAHTS DNA Clean Beads thoroughly by vortexing after equilibrating the magnetic beads to room temperature.
b. Pipette 45 µl of VAHTS DNA Clean Beads into 50 µl of the PCR amplification products. Mix the entire reaction system thoroughly by vortexing or gently pipetting up and down 10 times. Incubate at room temperature for 5 min.
c. Centrifuge the PCR tube briefly and place it on the magnetic stand to separate the magnetic beads from the solution. Wait until the solution becomes clear (about 5 min), and carefully remove the supernatant without disturbing the magnetic beads.
d. Keep the PCR tube on the magnetic stand. Add 200 µl of freshly prepared 80% ethanol to rinse the magnetic beads. Incubate at room temperature for 30 sec, and carefully remove the supernatant.

e. Repeat Step d for a second rinse.
f. Keep the PCR tube on the magnetic stand. Uncap the tube and air-dry the magnetic beads for 3 - 5 min.
g. After the beads are dried, remove the PCR tube from the magnetic stand and elute with 22 µl of ddH2O. Mix the magnetic beads thoroughly by vortexing or gently pipetting up and down 10 times, and incubate at room temperature for 5 min.
▲The products from Step h can be stored at -30 ~ -15°C. Avoid repeated freeze-thaw cycles during long-term storage.
h. Centrifuge the PCR tube briefly and place it on the magnetic stand to separate the magnetic beads from the solution. Wait until the solution becomes clear (about 5 min), and carefully pipette 20 µl of the supernatant into a new EP tube. Store at -30 ~ -15°C.

7-11/Library Quality Control

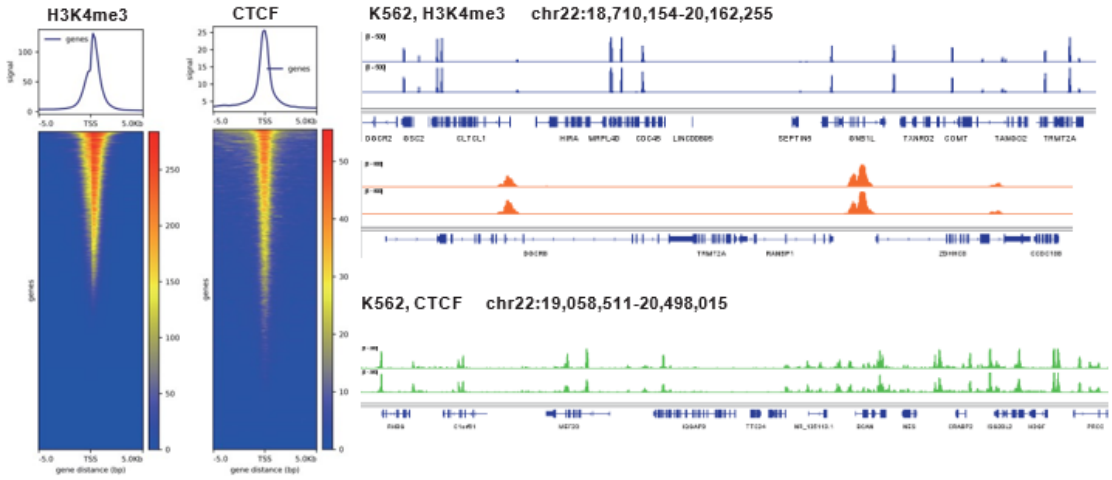
1.Library size distribution analysis:

The library size distribution analysis can be performed by devices based on electrophoretic separation such as LabChip GX, GXII, GX Touch (PerkinElmer); Bioanalyzer, Tapestation (Agilent Technologies); and Fragment Analyzer (Advanced Analytical). A CUT&RUN assay is performed for histone H3K4me3 using 100,000 K562 cells. The size distribution of the library obtained is presented in the figure below:



2.Sequencing data analysis, transcription start site (TSS) enrichment, and Integrative Genomics Viewer (IGV) views:

CUT&RUN assays are performed for histone H3K4me3 and transcription factor CTCF using 100,000 K562 cells. The TSS enrichment results and IGV views obtained are presented in the figure below:



8/FAQ & Troubleshooting

◇What species can CUT&RUN be applied to?

The CUT&RUN protocol is widely applicable for studying protein-DNA interactions in common mammalian cells. Yeast and plant cells should be treated before proceeding with the assay, but a low cell input amount is not recommended as this may lead to poor sequencing quality.

◇What are ConA magnetic beads mainly used for?

Concanavalin A (ConA)-coated magnetic beads bind to glycoproteins on the cell membrane, enabling the adsorption of cells and visualization of the cell handling process to reduce cell loss in the subsequent experimental steps.

◇Are CUT&RUN products only suitable for sequencing on Illumina platforms?

The adapters in VAHTS DNA Adapters for Illumina are specially designed for Illumina platforms. If another sequencing platform needs to be used, you should use amplification primers and adapters compatible with that platform.

◇Can the CUT&RUN kit be used with dual-indexed adapters?

Yes. The library preparation reagents provided in the kit are compatible with single- and dual-indexed adapters, which can be selected according to the sample quantity and sequencing requirements. For single-indexed adapters, we recommend VAHTS DNA Adapters for Illumina (Neo Biotech # NB-54-0066/ NB-54-0067/ NB-54-0070/ NB-54-0071/ NB-54-0072/ NB-54-0073); for dual-indexed adapters, we recommended VAHTS Multiplex Oligos Set 4/5 for Illumina (Neo Biotech # NB-54-0047/ NB-54-0048). Note the differences in the use of the two types of adapters.