GENE
CREATE

Animal Chromatin Immunoprecipitation (ChIP) Kit

Catalog#JKR23002A

Instruction Manual (For Two Groups)

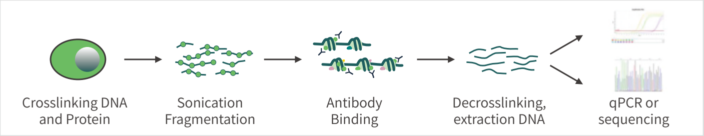
Sufficient reagents for 6 ChIP assays per kit.
Store at -20 & 4°C

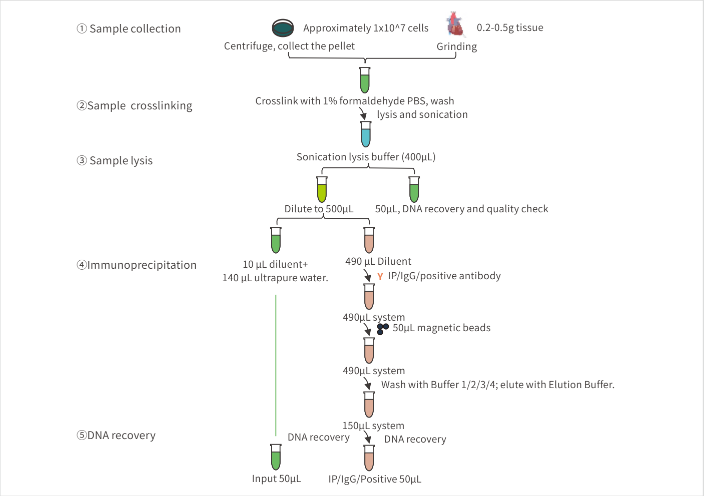
Table of Contents

1.Experimental Principle

Chromatin Immunoprecipitation (ChIP) is used to study whether proteins and DNA interact in vivo. This technique helps researchers determine what histone modifications occur at specific locations in the genome within the nucleus. By leveraging the specific binding of antibodies to antigens, DNA fragments bound to the target protein are precipitated, accurately reflecting regulatory proteins bound to DNA sequences.

2.Experimental Procedure





3.Kit Components

Box A					
Component	**Volume (6T)**	**Storage Temperature**	**Component**	**Volume (6T)**	**Storage Temperature**
5×PBS	7mL	4°C	ChIP Buffer	7mL	4°C
Glycine buffer	700μL	4°C	Protein A/G Magnetic Beads	350μL	4°C
Cell Lysis Buffer	3mL	4°C	Elution Buffer	1mL	4°C
Tissue Lysis Buffer	3mL	4°C	5M NaCl	150μL	4°C

Box B					
Component	**Volume (6T)**	**Storage Temperature**	**Component**	**Volume (6T)**	**Storage Temperature**
Wash Buffer 1	7mL	-20°C	Normal Mouse IgG	6 μL	-20°C
Wash Buffer 2	7mL	-20°C	Positive antibody (Histone-H3)	6 μL	-20°C
Wash Buffer 3	7mL	-20°C	5μM GAPDH primer (human)	40μL	-20°C
Wash Buffer 4	14mL	-20°C	5μM GAPDH primer (mouse)	40μL	-20°C
Proteinase K	48μL	-20°C	Protease Inhibitor (100×)	40μL	-20°C
Normal Rabbit IgG	6μL	-20°C	RNase A	24μL	-20°C

Note: 6T corresponds to 6 single immunoprecipitation experiments; the ChIP-qPCR procedure includes IgG, IP, and positive control (optional) groups, requiring 2-3T of reagents.

The kit does not include the following products:

- 37% formaldehyde or 16% formaldehyde
- DNA purification and recovery kit
- DNA library preparation kit
- Qubit fluorometer and quantification kit
- SYBR Green qPCR Mix

4. Operating Steps

4.1 Sample Crosslinking

Preparation: 1×PBS: Take 1mL of 5×PBS, add 4mL of ultrapure water, mix well;
1% Formaldehyde PBS Solution: Take 27μL of 37% formaldehyde solution, add 973μL of 1×PBS, mix well.

a Cell Sample

1) Cell Harvesting: Harvest approximately 1×10^7 cells, wash twice with pre-chilled PBS, 2mL each time, centrifuge at 1000g, 4°C for 5min, aspirate the liquid completely after the last wash.
2) Cross-linking: Add 1 mL of 1% formaldehyde PBS solution to resuspend and mix, then incubate with rotation at room temperature for 5 min;
3) Quenching cross-linking: Add 100 μL of Glycine Buffer, incubate with rotation at room temperature for 5 min, centrifuge at 1000g, 4°C for 5 min, and discard the supernatant;
4) Washing: Wash the cells twice, each time adding 2 mL of pre-cooled PBS to resuspend and mix, then centrifuge at 1000g, 4° C for 5 min to collect the cell pellet.

b Tissue samples

1) Grinding: Take 0.2-0.5g of fresh tissue or cryopreserved tissue stored at ultra-low temperature, place it in a pre-cooled mortar, add liquid nitrogen, and grind into a powder, then collect into an EP tube (for easily lysed internal organs such as liver and kidney, they can be cut into 1-2 mm small pieces using a grinding rod or surgical scissors);
2) Cross-linking: Add 1mL of 1% formaldehyde PBS solution, pipette to mix, and incubate with rotation at room temperature for 10min;
3) Cross-linking termination: Add 100μL Glycine Buffer, incubate with rotation at room temperature for 5min, centrifuge at 1500g, 4°C for 5min, and discard the supernatant;
4) Washing: Wash the precipitate twice, each time resuspending with 2 mL of pre-chilled PBS, then centrifuge at 1500g, 4°C for 5 min, and collect the tissue precipitate.

Note: The crosslinking temperature must always be kept below 25°C; formaldehyde concentration and crosslinking time vary for different sample types and can be adjusted according to established conditions.

4.2 Sample Lysis

Preparation: Add 445 μL of Cell Lysis Buffer (for cell samples) or Tissue Lysis Buffer (for tissue samples) to 5 μL of Protease Inhibitor (100X) and mix well (if slight precipitation or turbidity is observed in the Lysis Buffer during use, this is normal and it can be used after warming to room temperature or heating at 37°C).

1) Lysis: For cell samples, add 400 μL of Cell Lysis Buffer to the cross-linked pellet from the previous step and rotate-incubate at 4°C for 30 min, or lyse on ice for 30 min, vortexing once every 5 min; for tissue samples, add 400 μL of Tissue Lysis Buffer and rotate-incubate at 4°C for 40–60 min, or lyse on ice for 40–60 min, vortexing once every 5 min.
2) Sonication: Low-temperature sonication disruption (pre-experiment optimization of disruption conditions based on the type of sonicator is recommended; consult the manufacturer for reference conditions: 20% power, 5s on, 2s off, 6–10 min for cell samples, 15–30 min for tissue samples, all performed in an ice bath). After sonication, centrifuge at 10,000g, 4°C for 10 min, and collect the supernatant (this step can be paused, store samples at -20°C);

Note: Ultrasonic conditions need to be optimized for different cell and tissue types. It is recommended to pre-optimize ultrasonic conditions for various samples before each chip experiment. A gradient quality control with several ultrasonic durations can be performed: after running at the shortest reference time, take 10μL samples every 5 minutes for 1% agarose gel electrophoresis to determine DNA fragment size.

3) Reverse cross-linking: Take 50μL supernatant, add 100μL ultrapure water and 1μL RNase A, mix well and incubate at 37°C for 5 minutes. Then add 6μL 5M NaCl and 2μL Proteinase K, and incubate at 65°C for 3 hours or overnight.
4) DNA recovery: Use a DNA purification and recovery kit according to the manufacturer's instructions, and finally elute with 50μL ultrapure water (this sample can also serve as Input).
5) Quality Control: Determine DNA concentration using a Qubit fluorometer, and detect DNA fragment size by 1.5% agarose gel electrophoresis (optimal fragment range is 200-700bp for ChIP-qPCR and around 200-500bp for ChIP-seq).

4.3 Magnetic Bead Preparation (per IP reaction)

1) Take the Protein A/G Magnetic Beads from the 4°C refrigerator, invert several times to mix, and transfer 50μL to a 1.5mL EP tube;
2) Add 200μL of pre-chilled ChIP Buffer to resuspend the beads, place on a magnetic stand for 2min, aspirate and discard the supernatant , and repeat this step once , for a total of two washes.

4.4 Immunoprecipitation (per IP reaction)

According to experimental requirements, perform experimental grouping: for ChIP-qPCR, groups are Input, IgG, IP, and positive (optional) groups, requiring 2-3 IP reactions. For ChIP-seq, groups are Input and IP groups, requiring 1 IP reaction.

1) Based on the DNA concentration determined by quality testing, dilute the supernatant sample after sonication with ChIP Buffer to a final volume of 500μL (dilute histone target DNA concentration to 10-20μg/mL, transcription factor target DNA concentration to 20-40μg/mL). Take 10μL of the diluted sample, add 140μL of ultrapure water, and store as Input at -20°C for decrosslinking and recovery together with the enriched samples.
2) Take 490 μL of the diluted sample, add 3-5 μg of the target antibody (IP group) or 1 μL of isotype IgG (IgG group) or 3 μL of positive antibody (positive group), and incubate with rotation at 4°C for 3 hours or overnight;
3) Retrieve the incubated sample, centrifuge briefly for 3 seconds, and add it to the prepared magnetic bead tube, then incubate with rotation at 4°C for 2 hours;
4) Remove the sample from the silent mixer, place it on a magnetic stand for 2 minutes, and discard the supernatant;
5) Add 1 mL of Wash Buffer1 to resuspend the magnetic beads, place on a magnetic stand for 2 minutes, and discard the supernatant;
6) Add 1 mL of Wash Buffer2 to resuspend the magnetic beads, place on a magnetic stand for 2 minutes, and discard the supernatant;
7) Add 1 mL of Wash Buffer 3 to resuspend the magnetic beads, place on the magnetic stand for 2 min, and discard the supernatant;
8) Add 1 mL of Wash Buffer 4 to resuspend the magnetic beads, place on the magnetic stand for 2 min, discard the supernatant, repeat this step once, then briefly centrifuge for 3 sec, discard the supernatant, and collect the pellet.

4.5 Elution and Recovery

Preparation: Take the Elution Buffer out from 4°C and allow it to reach room temperature until completely dissolved (can be heated at 37°C to dissolve).

1) Add 150μL Elution Buffer to the magnetic bead pellet from the previous step, vortex to mix, incubate with rotation at room temperature for 15min, centrifuge briefly for 3S, place on a magnetic stand for 2min, and collect the supernatant;
2) Add 1μL Rnase A, mix, and incubate at 37°C for 5min. Then add 6μL 5M NaCl and 2μL Proteinase K, and incubate at 65°C for 3h (Input begins simultaneous operation at this step);
3) Proceed according to the instructions of the DNA purification and recovery kit, finally eluting with 50μL ultrapure water.

5. Experimental Analysis

5.1 ChIP-qPCR (Optional)

1) Reaction procedure: Add 2μL each of Input, IP, IgG, and positive control samples into PCR reaction wells, with three replicate wells per sample. Add remaining components according to the SYBR Green qPCR Mix instructions. After addition, label and centrifuge briefly for 10s, then place in the real-time PCR instrument for detection. Detailed sample loading is as follows (for positive controls, only GAPDH primers are required to confirm enrichment):

		1	2	3	4	5	6	7	8	9	10	11	12
GAPDH	A	Input	Input	Input	IgG	IgG	IgG	IP	IP	IP	阳性	阳性	阳性
Primer1	B	Input	Input	Input	IgG	IgG	IgG	IP	IP	IP			
Primer2	C	Input	Input	Input	IgG	IgG	IgG	IP	IP	IP			
...	...	Input	Input	Input	IgG	IgG	IgG	IP	IP	IP			

2) Result Calculation

ΔCt [normalized ChIP] = (Ct [ChIP] - (Ct [Input] - Log2 (Input Dilution Factor)) Input Dilution Factor = (fraction of the input chromatin saved)^-1

Note: In our company's projects and kits, Input Dilution Factor = 50, log2(50) = 5.644

ΔΔCt [ChIP/NIS] = ΔCt [normalized ChIP] — ΔCt [IgG]

Fold Enrichment = 2∧ (—ΔΔCt [ChIP/NIS])

%Input = 2%×2^(Ct[Input] - Ct[ChIP])

5.2 ChIP-seq (Optional)

1) Library construction: Take an appropriate amount of Input and IP samples, and proceed according to the DNA library preparation kit instructions.

Library Quality Control

Sequencing

Bioinformatics Analysis

6. Frequently Asked Questions

Q1: How to Explore Ultrasonic Conditions

1) Contact Ultrasonic Instrument: Take a 1.5 mL EP tube, add 0.4-0.6 mL of liquid, place the probe at the center of the EP tube, about two- thirds below the liquid surface. Set the ultrasonic time to 5 seconds, gradually increase the power until bubbling begins—this power is the maximum ultrasonic power. Based on this, set three different power gradients and time gradients for exploration.
2) Non-contact: Can be explored as recommended by the manufacturer.

Q2: Fragments after sonication do not meet requirements

1) There are fragments that meet requirements and also large fragments that cannot be sonicated and are relatively concentrated, possibly due to excessively high crosslinking temperature. Always keep the temperature below 25°C during crosslinking, especially in summer when temperatures are high; reagents and samples can be placed at the air conditioning vent for a period before operation.
2) Fragments are highly concentrated and less than 200bp, indicating unsuitable sonication power and time; pre-experiments are needed to explore optimal sonication conditions.

Q3: No difference in Ct values between IP and IgG samples

1) Antibody did not enrich DNA; try replacing the antibody.
2) If the IgG background is too high, increase the number of washes or reduce the DNA input in the immunoprecipitation step.
3) Incorrect binding site prediction requires redesigning the primers.

Q4: Abnormal melting curve

1) A non-single peak in the melting curve may indicate non-specific amplification or primer dimer formation, requiring primer redesign.

Q5: Sample DNA concentration is very low, below 10 ng/μL

1) Insufficient sample input: consider increasing the initial sample input, especially for tissues like muscle or heart.
2) Incomplete lysis: sample is over-crosslinked or insufficiently ground.
3) For difficult-to-lyse samples, after adding LysisBuffer, rapidly freeze at -80°C and thaw at 37°C, repeating the freeze-thaw cycle 3 times.

Simplified Operation Manual

1. Sample Crosslinking

1) 1×10^7 cells or liquid nitrogen-ground tissue powder, add 1mL of 1% formaldehyde PBS solution, rotate and incubate at room temperature for 5/10min, depending on sample type;
2) Add 100μL Glycine Buffer, rotate and incubate at room temperature for 5min, centrifuge at 1000g, 4°C for 5min, discard supernatant;
3) Wash twice with 1mL pre-cooled PBS.

2. Sample Lysis

1) Add 400μL Lysis Buffer (containing Protease Inhibitor) and rotate-incubate at 4°C for 30-60min, or lyse on ice for 30-60min, depending on the sample type;
2) Perform low-temperature sonication, then centrifuge at 10000g, 4°C for 10min, and collect the supernatant (reference conditions: 20% power, 5S on, 2S off, sonicate cell samples for 6~10min, tissue samples for 15~30min, all in an ice bath);
3) Take 50μL of supernatant, add 100μL ultrapure water and 1μL RNase A, mix and incubate at 37°C for 5min, then add 6μL 5M NaCl and 2μL Proteinase K, and incubate at 65°C for 3h or overnight;
4) Recover DNA, finally elute with 50μL ultrapure water;
5) Measure the concentration of recovered DNA, and detect DNA fragment size by 1.5% agarose gel electrophoresis.

3. Magnetic bead preparation

1) Take 50 μL of Protein A/G Magnetic Beads;
2) Wash the beads twice with 200 μL of pre-chilled ChIP Buffer.

4. Immunoprecipitation

1) Dilute the sample with ChIP Buffer, take 10 μL of the diluted sample, add 140 μL of ultrapure water, and store as Input at -20°C;
2) Take 490 μL of the diluted sample, add 3-5 μg of the target antibody (IP group) or 1 μL of same-species IgG (IgG group) or 3 μL of positive antibody (positive group), and incubate with rotation at 4°C for 3 hours or overnight;
3) After incubation, add to the prepared magnetic bead tube and incubate with rotation at 4°C for 2 hours;
4) Wash the magnetic beads once each with Wash Buffer1, Wash Buffer2, Wash Buffer3, and twice with Wash Buffer4.

5. Elution and Recovery

1) Vortex and mix with 150 µL Elution Buffer, then incubate with rotation at room temperature for 15 minutes;
2) Add 1µL of Rnase A, mix and incubate at 37°C for 5 minutes. Then add 6µL of 5M NaCl and 2µL of Proteinase K, and incubate at 65°C for 3 hours (Input begins simultaneous operation at this step);
3) DNA recovery, elute with 50µL of ultrapure water.

6. Experimental analysis

1) ChIP-qPCR (optional)
2) ChIP-seq (optional)

GENE
CREATE

WUHAN GENECREATE BIOLOGICAL ENGINEERING CO.,LTD.

Address： Wuhan Shengzhiyuan Biotechnology Innovation Industrial Park,
North Shendun 5 th Road, Jiangxia District, Wuhan City, Hubei Province.
Phone: 027-87960366
Email: marketing@genecreate.com
Website: www.genecreate.cn