ChIP Assay Kit (Protein A/G Magnetic Beads）

Product Description:

ChIP (chromatin immunoprecipitation) is an experimental technique used to study protein-DNA interactions, allowing researchers to identify binding sites in the genome of specific proteins, typically transcription factors or histone modifications. The basic steps of a ChIP experiment include cross-linking, lysis, immunoprecipitation, reverse fixation, DNA extraction, and DNA analysis. Common analysis methods include magnetic bead method and agarose method, the conventional agarose method requires long-term centrifugation, while the magnetic bead method does not need centrifugation, only needs a short time of magnetic separation, which can effectively avoid the mechanical force effect caused by centrifugation on the immune complex, and fully ensure the natural state of the immune complex.

Protein A/G Magnetic Beads are used to immunoprecipitate a wide range of antibodies, including mouse IgG1, IgG2a, IgG2b, IgG3, IgA, rat IgG1, IgG2a, IgG2b, IgG2c, rabbit IgG, rabbit and goat polyclonal Abs, as well as human IgG1, IgG2, IgG3 and IgG4. At the same time, the non-specific binding of Protein A+G Agarose presaturated with Salmon Sperm DNA and the target genomic DNA is greatly reduced. If the ChIP product is used for chromatin immunoprecipitation sequencing (ChIP-Seq), possible Salmon Sperm DNA data needs to be removed from the data.

Pre-mixed control primers (Control Primers) are provided for amplification of some of the corresponding sequences of human GAPDH: 5'-TACTAGCGGTTTTACGGGCG-3' and 5'-TCGAACAGGAGGAGCAGAGAGCGA-3'.

The ChIP Assay Kit can be used for routine chromatin immunoprecipitation, resulting in a total of 22 samples.

Composition and storage conditions

Components	K4626-22 T
Protein A/G Magnetic Beads/Salmon Sperm DNA	3 mL
Glycine Solution (10X)	30 mL
ChIP Dilution Buffer	48 mL
Low Salt Immune Complex Wash Buffer	24 mL
High Salt Immune Complex Wash Buffer	24 mL
LiCl Immune Complex Wash Buffer	24 mL
TE Buffer	48 mL
0.5 M EDTA	250 µL
5 M NaCl	500 µL
1 M Tris, pH 6.5	500 µL
SDS Lysis Buffer	10 mL
Control Primers (5 µM each)	0.1 mL
Store all the components at 4°C for 1 year.	

Experimental manipulation

1. Optimization of sample sonication conditions

1.1 Prepare an appropriate amount of ice bath pre-chilled PBS, and an appropriate amount of protease or phosphatase inhibitor Cocktail (K1007 or K1015). Warm the SDS Lysis Buffer in a proper warm bath so that the SDS in it is fully dissolved and mix well. *[Note] The final concentration of PMSF is usually 1 mM, and it should be noted that the aqueous solution of PMSF must be freshly prepared, and its half-life in the aqueous phase is about 30 minutes.*

1.2 Cells were cultured in a 10 cm cell culture dish with a volume of 10 mL of cell culture medium. At the time point when binding of the protein of interest to genomic DNA is expected, an appropriate amount of formaldehyde is added directly to the cell culture medium and gently mixed until the final concentration is 1%. Incubate at 37°C for 10 min to cross-link the protein of interest and the corresponding genomic DNA. For example, for a routine 10 mL of cell culture medium per 10 cm dish, 270 µL of 37% formaldehyde would be added. Please try to use high-quality formaldehyde within the expiration date. Cells can also be cultured in 6 cm cell culture dishes, and the amount of solution used should be adjusted accordingly.

1.3 Add 1.1 mL of Glycine Solution (10X) and mix gently. Leave at room temperature for 5 min.

1.4 Place the Petri dish with the cell sample on an ice bath. Aspirate the culture medium containing formaldehyde and glycine and try to keep no liquid residue.

1.5 During the 5 min at room temperature described above, dilute the protease or phosphatase inhibitor

Cocktail with ice bath pre-chilled PBS and add to the Petri dish.

1.6 Add 5-10 mL of pre-chilled protease inhibitor-containing PBS in an ice bath, wash the cells, and aspirate the liquid, trying to keep no liquid carryover.

1.7 Add 5-10 mL of PBS with protease inhibitors pre-chilled in an ice bath and wash the cells further, aspirating the liquid, and trying to keep no liquid carryover.

1.8 Add 1 mL of pre-chilled PBS containing protease inhibitors in an ice bath, scrape off the cells with a cell scraper, and collect them into a centrifuge tube. If the cells can be pipetted down with a gun, they can also be piped with a gun. Cells are counted and aliquoted into approximately 1 million cells per tube.

1.9 Centrifuge at 800-1,000 g for 1-2 min at 4°C to pellet cells thoroughly. If the precipitation is found to be insufficient, the centrifugation time can be extended appropriately. Aspirate the supernatant to minimize liquid residue.

1.10 Prepare an appropriate amount of SDS Lysis Buffer containing protease inhibitors. The 1,000,000 cell pellet from the previous step was resuspended with 0.2 mL of SDS Lysis Buffer containing 1 mM protease inhibitors.

1.11 Incubate on an ice bath for 10 min to fully lyse the cells.

1.12 Sonication to shear genomic DNA so that most of the DNA breaks into 200-1,000 bp in size, preferably if most of it can be controlled to 400-800 bp. Care must be taken to keep the sample in an ice bath and at a low temperature during ultrasound. The effect of sonication shearing can be detected with conventional DNA agarose gel electrophoresis after subsequent de-crosslinking. The conditions of sonication can usually be set to 10 seconds per sonication, with a 10 second stop for a total of about 5-30 times, with an actual power of 10-40 W, using a 2-3 mm ultrasound head. The specific settings of different sonication instruments may be different, when exploring the ultrasound conditions, you can first fix other conditions, first determine how long each ultrasound and pause (it is recommended to try 10 seconds per ultrasound for 10 seconds or 10 seconds for 20 seconds for ultrasound) will not cause significant heat and no foam production, and then explore different ultrasound times (such as 5, 10, 20, or 30 times), usually the higher the actual power, the less total ultrasound time. Until a suitable number of ultrasounds is found, most of the genomic DNA can be broken into 200-1,000 bp in size. It should be noted that the volume of each sonication and the cell type and dosage should be fixed, otherwise a relatively fixed ultrasound condition cannot be used for subsequent experiments.

[Note] When measuring the size of genomic DNA after ultrasound, if a safe dye such as NA-Red, NA-Green, Gel-Red or Gel-Green is added to an agarose gel or a DNA loading buffer containing such a safe dye is used, the SDS will combine with such dyes to form abnormal bands during electrophoresis, which are usually about 500-1,000 bp, which will affect the determination of

genomic DNA size after ultrasound. It is recommended to use the method of "staining the agarose gel after electrophoresis" to detect the band size, which will not cause abnormal bands, does not affect the judgment of genomic DNA size after ultrasound, and the band size is more accurate.

1.13 Add 8 μL of 5 M NaCl to 0.2 mL of sonicated sample and mix. Heat at 65°C for 4 h to remove cross-links between protein and genomic DNA.

1.14 Add an equal volume of Tris-balanced phenol, vortex mix vigorously, and then centrifuge at 4°C, 12,000 g or so for 5 min. Aspirate the supernatant into another centrifuge tube.

1.15 Equal volume chloroform was added, vortex was vigorously mixed, and then centrifuged at 4°C at about 12,000g for 5 min. Aspirate the supernatant into another centrifuge tube.

1.16 A small amount of liquid obtained by phenol chloroform extraction or DNA purification kit, 5-10 μL for phenol chloroform extraction product and 2-5 μL for DNA purification kit purification product were taken for agarose gel electrophoresis to observe the shearing effect of sonication on genomic DNA.

2. Chromatin immunoprecipitation

2.1 After the sonication conditions of the samples were optimized, the samples to be tested were performed according to steps 1.1-1.11, and sonicated with reference to step 1.12.

2.2 Subsequently, for sonicated samples, centrifuge at 12,000-14,000 g for 5 min at 4°C. Add the supernatant (approximately 0.2 mL) to a 2 mL centrifuge tube and place in an ice bath.

2.3 Prepare an appropriate amount of ChIP Dilution Buffer containing 1 mM protease inhibitors. Add 1.8 mL of ChIP Dilution Buffer containing 1 mM protease inhibitor to dilute the sonicated sample to a final volume of 2 mL.

2.4 Remove 20 μL of sample as input for subsequent testing. For the remaining nearly 2 mL samples, 70 μL of Protein A+G Magnetic Beads/Salmon Sperm DNA was added and mixed slowly at 4°C for 30 minutes. The purpose of this step is to reduce the non-specific binding of Protein A/G Magnetic Beads/Salmon Sperm DNA to the protein of interest or the DNA sequence of interest. It is then placed on a magnetic stand for 10 seconds to transfer the supernatant to a new 2 mL tube. *Note: Removal of non-specific binding is optional.*

2.5 Add an appropriate amount of primary antibody, and the dosage of primary antibody can refer to the instructions of the antibody. If the dilution for ChIP is not given in the description of the antibody, refer to the dilution ratio for common immunoprecipitation. The usual amount of primary antibody is 0.5 to 1 μg. Mix slowly at 4°C with rotation or swing overnight or at least 4 h or more. No antibody can be added as a negative control, or an unrelated antibody can be used as a negative control, and a solution without a cell sample can be used as a blank control.

2.6 Add 80 μL of Protein A+G Magnetic Beads/Salmon Sperm DNA and mix slowly at 4°C for 60

minutes with slow rotation or shaking to precipitate the protein recognized by the primary antibody or the corresponding complex.

2.7 Place on a magnetic stand for 10 sec to remove the supernatant without touching the beads. The beads were washed sequentially with the following solution, each wash was 1 mL, slowly rotated or swung at 4°C for 3-5 minutes each time, and then placed on a magnetic stand for 10 seconds to separate, carefully removing the supernatant without touching the beads.

2.8 Wash once with Low Salt Immune Complex Wash Buffer.

2.9 High Salt Immune Complex Wash Buffer wash once.

2.10 LiCl Immune Complex Wash Buffer is washed once.

2.11 TE Buffer is washed twice.

[Note] The precipitate obtained after all the above washing steps can be used for PCR amplification of the gene sequence of interest, detection of the gene sequence of interest with Southern, or for Western detection.

3. PCR amplification of the gene sequence of interest (if the ChIP product is used to detect the gene sequence of interest)

3.1 Prepare an appropriate amount of Elution buffer (1% SDS, 0.1 M $NaHCO_3$) freshly.

3.2 After step 2.11, i.e., after all wash steps, add 250 μL of Elution buffer. Vortex mixes, turn or swing at room temperature to continue eluting for 3-5 min.

3.3 Place on a magnetic stand for 10 sec and transfer the supernatant to a new centrifuge tube. Add 250 μL of Elution buffer to the pellet. Vortex mixes, turn or swing at room temperature to continue eluting for 3-5 min.

3.4 Place on a magnetic stand for 10 sec and remove the supernatant. and the supernatant merge obtained in the previous step, step 3.3. Total approximately 500 μL of supernatant.

3.5 Add 20 μL of 5M NaCl to 500 μL of supernatant and mix. Heat at 65°C for 4 h to remove cross-links between protein and genomic DNA. For the 20 μL sample obtained as input in step 2D, 1 μL of 5M NaCl was added, mixed well, and heated at 65°C for 4 hours, also to remove crosslinks between protein and genomic DNA. Once this step is complete, you can proceed to the next step, or you can cryostore at -20°C and continue with the next step.

[Note 1] At this time, the sample is ready for PCR, and you can try to use 1, 2, 5, or 10 μL of the sample as a template for PCR detection of the gene of interest. The effect of PCR at this point is related to the amount of DNA that may be precipitated, and whether the overall PCR amplification system is easy to amplify the gene of interest. If PCR is found to be suboptimal, consider purifying and concentrating the sample with a subsequent purification step prior to PCR testing.

[Note 2] In general, it is recommended to perform a follow-up purification followed by a PCR test, but Input usually does not need to perform a follow-up purification step.

3.6 Add 10 μL of 0.5 M EDTA, 20 μL of 1 M Tris pH 6.5, and 1 μL of 20 mg/mL proteinase K to approximately 520 μL of sample. Incubate at 45°C for 60 min after mixing.

3.7 Equilibrated phenol was added in equal volume Tris, vortex was vigorously mixed, and then centrifuged at 4°C, around 12,000 g for 5 min. Aspirate the supernatant into another centrifuge tube.

3.8 Add an equal volume of chloroform, mix vigorously with vortex, and centrifuge at 4°C at around 12,000 g for 5 minutes. Aspirate the supernatant into another centrifuge tube.

3.9 Add 20 μg of glycogen or yeast tRNA, 1/10 volume of 3 M NaAc, pH 5.2, and 2.5 volumes of absolute ethanol. After mixing, the precipitation should not be less than 1 hour at -70°C, or more than 8 hours at -20°C.

3.10 Centrifuge at 12,000-14,000 g for 10 min at 4°C, carefully aspirating most of the supernatant without touching the pellet.

3.11 Add approximately 1 mL of 70% ethanol to wash the pellet. Centrifuge at 4°C, 12,000-14,000 g for 10 min, carefully aspirate most of the supernatant without touching the pellet.

3.12 Centrifuge at 12,000-14,000 g for 1 min at 4°C and aspirate the residual liquid very carefully.

3.13 Resuspend the DNA pellet with a small amount of TE or water for PCR detection of the gene of interest. It is best to design 2 sets of primers for PCR, and the corresponding PCR conditions can be explored in advance with Input as a template, and a group of primers with better results can be selected for the final PCR detection. Rarely, when the PCR band is too weak, two rounds of amplification can be performed using nested PCR. After amplification of the band of interest with conventional PCR, the sample can be used directly for qPCR quantification, or it can be used for library preparation and high-throughput sequencing (ChIP-seq).

Notes

1. Do not cryopstore Protein A+G Agarose/Salmon Sperm DNA, other solutions can be frozen at -20°C for longer storage.
2. Primary antibodies for ChIP, 37% formaldehyde, PBS, PMSF, Elutioin (1% SDS, 0.1 M NaHCO3), proteinase K, Glycogen or tRNA, Tris balanced phenol, chloroform, 95% ethanol, 70% ethanol, 3 M NaAc (pH 5.2), cell scraper or cell spatula, and magnetic stand.
3. You need to bring your own sonicator, also known as ultrasonic pulverizer or ultrasonic cell pulverizer.
4. When using formaldehyde, operate in a fume hood.

5. This product is for scientific research purposes only.

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