Signosis
Innovative Plate Assay Solutions

H3 Snapshot ChIP assay Kit

Catalog Number CA-0131 **(For Research Use Only)**

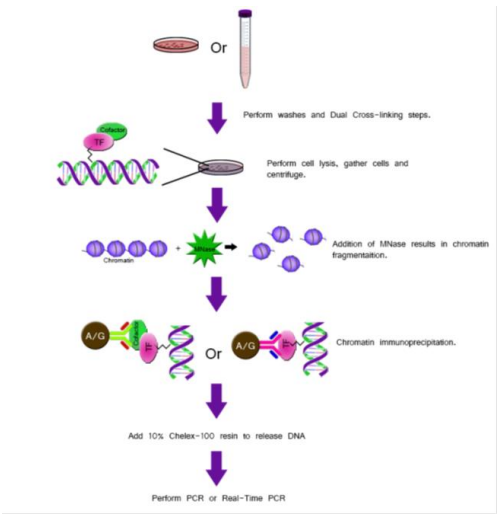
Introduction

H3 is involved with the structure of the nucleosomes of the 'beads on a string' structure. Histone H3 is an important protein in the emerging field of epigenetics, where variable modification states are thought to play a role in the dynamic and long term regulation of genes.

Unlike electrophoretic mobility shift assays detecting the binding of specific TFs present in cell lysates to DNA sequences in vitro, chromatin immunoprecipitation (ChIP) assay is enable the analysis of the association of H3 with specific promoters in vivo and provides a snapshot of how a regulatory TF or co-factor affects the expression of a single gene or a variety of genes at the same time. With several improvements, Signosis' H3 ChIP assay is able to efficiently measure the interaction of a specific TF or associated cofactors with target promoters in human and mouse samples.

Principle of the assay

The ChIP assay typically comprises four steps: (1) cross-linking proteins to DNA; (2) chromatin fragmentation; (3) protein precipitation; and (4) target identification and quantitation. The initial cross-linking is to insure that protein-DNA complexes remain associated through the subsequent steps. Formaldehyde is the most commonly used reagent for the cross-linking of proteins that are directly bound to DNA, such as transcription factors and histones, but not for coactivators and corepressors indirectly associated with DNA due to short spacer arm. Several improvements in Signosis' ChIP assay are highlighted as follows: firstly, the dual cross-linking, formaldehyde (short space arm) and second cross-linking reagent (long space arm), is introduced to ensure the detection of both TFs and associated cofactors. Secondly, DNA is broken down to mononucleosomes and 500bp in size by MNase digestion, which enhances greatly the sensitivity and reproducibility. The cross-linked protein-DNA complexes are subsequently pulled down by a specific antibody and ChIP grade protein A/G magnetic beads. The last improvement is to release DNA from cross-linked proteins with one-step chelex-100 without tedious proteinase K digestion and further purification, significantly increasing the assay efficiency.



Materials provided with the kit

- H3 antibody
- Crosslinker reagent (longer space arm)
- 2X Cross linker buffer
- 1X Cell lysis buffer
- 1X Nuclei cleanup solution
- 1X MNase reaction buffer
- MNase
- 2X ChIP assay buffer
- 10X EDTA
- Protein A/G Magnetic beads
- 1X High salt wash buffer
- TE buffer
- 10% Chelex-100 solution
- 200X Proteinase inhibitor
- 200X DTT
- H3 control primer

Material required but not provided

- 1X PBS
- 37% Formaldehyde
- 1M Glycine
- DMSO
- Magnetic stand
- Rabbit or mouse IgG
- PCR thermocycler
- Sonicator

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- Rocker platform
- Heat block

Reagent preparation before starting experiment

- Culture and treat 4-6x10^6 cells for each experiment. This number of cells generates one chromatin preparation that can be used for 2 immunoprecipitation.
- For making crosslinker solution, dissolve 2mg of crosslinker in 20µl DMSO for each reaction, and add to 5ml 1X Crosslinker buffer and mix immediately to avoid aggregation. Prepare just before use and do not store in solution. Calculate the amount of crosslinker and volume of DMSO you need depending on how many reactions in one-time experiment.
- Unused crosslinker reagent in powder is stored at 4°C with tightly closed cap.
- Prior to use, prepare each working buffer by following the product insert.

Assay procedure

Dual cross-linking

For adherent cells, the cross-linking step can be performed on a plate; for suspension cells, the cells need to be collected first and the cross-linking step can be performed in a 15ml tube.

1. Wash the cells two times with cold 1X PBS.
2. Add 5ml crosslinker solution to the cells and incubate for 20 minutes at room temperature with gentle rocking.
3. Discard the crosslinker solution and wash 3 times with cold 1X PBS.
4. Add 5ml 1% Formaldehyde diluted in 1X Crosslinker buffer and incubate for 10 minutes at room temperature with gentle rocking.
5. To stop crosslinking reaction, add 325µl of 1M glycine and incubate for 10 minutes with gentle rocking at room temperature.
6. Remove the solution and wash the cells with cold 1X PBS three times. Remove as much PBS as possible after the last wash.
   Note: If you wish to stop the protocol and continue later, freeze the cells at -80°C.

Lysis of the cells and fragmentation

1. To lyse cells, add 1ml of 1X cell lysis buffer with gentle rocking for 10 minutes on ice.
2. Scrap cells off the plate (only for adherent cells), collect in a microcentrifuge tube, and centrifuge at 3000 rpm for 5 min to pellet nuclei.
3. Discard the supernatant and resuspend the pellet in 500ul 1X MNase reaction buffer by gently pipetting up and down until this nuclei suspension achieves a milky opalescent luster.
4. Add 500ul 1X Nuclei cleanup buffer in a microcentrifuge tube and then apply nuclei suspension to the top of cleanup solution and don't mix.
   Note: Some mixing will occur naturally at the interface.
5. Centrifuge for 5 minutes at 3000 rpm to pellet nuclei through.
6. Remove the buffer and resuspend the pellet in 200µl 1X MNase reaction buffer by gently pipetting up and down. The suspension should look like the way described in Step 3 (note: it is very important to resuspend the nuclei evenly in solution)
7. Add 6ul of MNase (20U/ul), mix by inverting tube several times and incubate for 30 minutes at room temperature with gentle shaking during incubation.
8. Stop the digestion by adding 20µl 10X EDTA
9. Add 200ul 2X ChIP Assay buffer and resuspend the pellet in the buffer by gently pipetting up and down. And incubate on ice for 10 minutes.
10. Briefly sonicate the sample for 10 seconds on ice with 10-12 kHz setting or similar setting.
11. Incubate the sample for 10 seconds on ice.
12. Repeat steps 10-11 two more times. This step will help to completely lyse the cells.
13. Centrifuge for 5 minutes at 10,000 rpm to clear lysate.
14. Carefully transfer the supernatant to a new tube.

Chromatin immunoprecipitation.

1. To setup IP, transfer 200ul of lysate in a 1.5ml tube and add 2ug antibody of interests. Take another 200ul of lysate and add 2ug of matching rabbit or mouse IgG as negative IP control or add another antibody. Incubate 3 hours to overnight at 4°C with rotation. *Store remaining 50ul lysate at -80°C for future usage and for total input control and PCR positive template.*
2. Wash 5µl Protein A/G Magnetic beads in 1ml of 1X ChIP buffer in a new tube by placing the tube on a magnetic stand for 30 seconds and then discard the buffer.
3. Transfer 200µl sample-antibody IP mixture from Step1 to the tube with magnetic beads and incubate at 4°C for 1 hour.

4. Transfer the bead mixture to another **fresh** tube. And place on the magnetic stand for 30 seconds and discard the solution.
   This step helps reduce background.
5. Add 1 ml 1X ChIP Assay buffer, mix well and place the tube on a magnetic stand for 30 seconds and discard the buffer.
6. Repeat steps 4 –5 one more time.
7. Wash the beads by adding 1ml 1X high salt buffer, mix well and place the tube on a magnetic stand for 30 seconds and discard the buffer.
8. Wash the beads by adding 1ml of TE buffer, mix well and **transfer the entire solution to a fresh tube.**
9. Place the new tube on a magnetic stand for 30 seconds and discard the buffer.
10. Add 50ul TE buffer to resuspend the magnetic beads and transfer to any type of tube matching the thermocycler in your lab (PCR tube, 0.5ml tube, or 1.5ml tube).
    This optional step is for easier handling on next heating procedure with PCR machine. You also can use common heat block or water bath.
11. Add 50ul 10% chelex-100 resin and mix well with magnetic bead solution.
12. Incubate the samples in a thermocycler with the following program:
    55°C for 1 hour
    98°C for 15 minutes
    37°C for 1 minute
    98°C for 15 minutes
    22°C for 5 minutes
13. Briefly centrifuge the tubes or place the tube on magnetic stand and let chelex-100 resin settle.
14. Carefully transfer the supernatant to a new tube. *NOTE: Avoid pipetting the beads and resin.*
15. Perform PCR with supernatant.
16. Perform PCR with 2µl Oct4 control primer in 40ul reactions for 35 cycles.