



Snapshot ChIP assay Kit

Catalog Number CA-0001

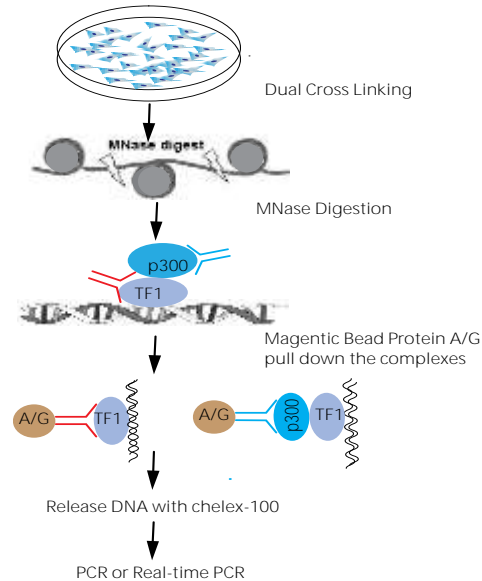
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Introduction

The differential gene expression depends not only on the binding of specific transcription factors (TFs) to discrete promoter elements but also on expression and interaction of co-activators with their associated TFs. The interactive cofactor complexes allow the functional integration of multiple TFs and pathways facilitating the formation of distinct biological programs. 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 a specific TF or co-factor 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' ChIP assay is able to efficiently measure the interaction of a specific TF or associated cofactors with target promoters.

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 following 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

- Crosslinker reagent (longer space arm)
- 2X Cross linker buffer
- 1X Cell lysis buffer
- 1 X 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

Material required but not provided

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

- Rocker platform
- Heating blocker

Reagent preparation before starting experiment

- Culture and treat $4-6 \times 10^6$ cells for each experiment. This number of cells generate one chromatin preparation that can be used for 2 immunoprecipitation.
- For make crosslinker solution, dissolve 2mg of crosslinker in 20 μ l 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.
- Prepare the working buffers before using following the product inset.

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 325 μ l 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 well resuspend the pellet in 500 μ l 1X MNase reaction buffer by gently pipetteing up and down until this nuclei suspension looks like a milky opalescent luster.

4. Add 500 μ l 1X Nuclei cleanup buffer in a microcentrifuge tubes and then apply nuclei suspension to the top of cleanup solution and Don't mix, but allow some mixings to occur at the interface.
5. Centrifuge for 5 minutes at 3000 rpm to pellet nuclei through.
6. Remove the buffer and resuspend the pellet in 400 μ l 1X MNase reaction buffer by gently pipetteing 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 6 μ l of MNase (20U/ μ l), mix by inverting tube several times and incubate for 30 minutes at room temperature with gently shaking during incubation.
8. Stop the digestion by adding 40 μ l 10X EDTA
9. Add 200 μ l 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 totally 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 200 μ l of lysate in a 1.5ml tube and add 2 μ g antibody of interests. Take another 200 μ l of lysate and add 2 μ g 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 50 μ l lysate at -80°C for future usage and for total input control and PCR positive template.*
2. Wash 5 μ l 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 discarding the buffer.
3. Transfer 200 μ l 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 6 –7 one more time.
7. Wash the beads by adding 1ml 1X high salt buffer, mix well and placing the tube on a magnetic stand for 30 seconds and discarding 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 and 1.5ml tube).
This step is optional for easier handling on next heating procedure with PCR machine. You also can use common incubator 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:
1hour at 55°C
15 min at 98°C
1min at 37°C
15 min at 98°C
22°C for 5 min
13. Briefly centrifuge the tubes or place the tube on magnetic stand and let chelex-100 resin settled.
14. Carefully transfer the supernatant to a new tube. *NOTE: Do not get the beads and resin in.*
15. Perform PCR with supernatant.
16. Perform PCR with 2ul control primer in 40ul reactions for 35 cycles.