Signosis
Innovative Plate Assay Solutions

Snapshot ChIP assay Kit

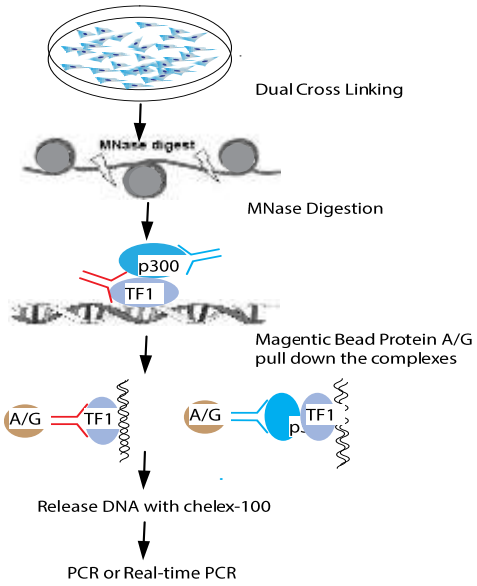
Catalog Number CA-0001 **(For Research Use Only)**

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

- Cross linking reagent (longer space arm)
- MNase
- 10XCross-linking buffer
- 10X Cell lysis buffer
- 10X MNase reaction buffer
- 2X ChIP buffer
- Protein A/G Magnetic beads
- 2X high salt wash buffer
- 10X TE buffer
- 10% Chelex-100 solution
- Proteinase inhibitor
- DTT

Material required but not provided

- 1X PBS
- Formaldehyde
- 1M Glycine
- DMSO
- Magnetic stand
- Rabbit or mouse IgG
- PCR thermocycler
- Sonicator
- Rocker platform
- Heating blocker

Reagent preparation before starting experiment

- **Adherent cell culture**
  Seed cells in 1X 100 mm^2 plate with 80 to 90% confluency for one ChIP reaction.
- Dissolve 1mg of crosslinker in 10µl DMSO and then immediately add in 10ml 1X Crosslinker buffer to avoid aggregation. This makes enough crosslinking reagent for 2X 100mm^2 plates. (Prepare just before use and do not store in solution. Unused crosslinker is stored at 4°C with tightly closed cap.)
- **1ml 1X cell lysis buffer** (for one reaction)
  100µl 10x cell lysis buffer
  890µl ddH2O
  5µl 200X protease inhibitor
  5µl 200X DTT
- **1.2ml 1X MNase reaction buffer** (for one reaction)
  120µl 10 x MNase reaction buffer
  1074µl ddH2O
  6µl 200X DTT
- **5ml 1X ChIP buffer** (for one reaction)
  2.475ml 2X ChIP buffer
  2.475ml ddH2O
  25µl 200X DTT
  25µl 200X proteinase inhibitor
- **1ml 1X High salt buffer** (for one reaction)
  495ul 2X High salt buffer
  495ul ddH2O
  5µl 200X DTT
  5µl 200X proteinase inhibitor
- **1ml 1X TE** (for one reaction)
  100ul 10X TE buffer
  890ul ddH2O
  5µl 200X DTT
  5µl 200X proteinase inhibitor

Notes: use and all of 1X buffer must be kept on ice before use.

Assay procedure

Dual cross-linking

1. Wash the cells two times with cold 1X PBS.
2. Add 5ml crosslinker solution to the cells and incubate for 45 minutes at room tempaterature with gentle rocking.
3. Discard the crosslinker reagent and wash 3 times with cold 1X PBS.
4. Add 5ml 1% Formaldehyde diluted in 1X Crosslinking buffer and incubate for 10 minutes at at room tempaterature with gentle rocking.
5. To stop crosslinking reaction, add 325µl of 1M glycine for 10 minutes with gentle rocking at at room tempaterature.
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 at 4°C for 10 mintues on ice.
2. Scrap cells off the plate, collect in a microcentrifuge tube, and centrifuge at 5000 rpm for 5 min to pellet nuclei.
3. Dicard the supernatant and resuspend the pellet in 1ml 1X MNase reaction buffer.
4. Centrifuge for 5 minutes at 3000 rpm to pellet nuclei.
5. Remove the buffer and resuspend the pellet in 150µl 1X MNase reaction buffer.
6. Add 0.5µl of MNase (1000U), mix by inverting tube several times and incubate for 20 minutes at 37°C and tap the tube every 3 – 5 minutes during incubation.
7. Stop the digestion by adding 30µl 50X EDTA.
8. Incubate the tube on ice for 5 minutes.
9. Sonicate the sample for 20 seconds on ice with 10-12 kHz setting.
10. Incubate the sample for 20 seconds on ice.
11. Repeat steps 9-10 two more times.
12. Centrifuge for 5 minutes at 10000 rpm to clear lysate.
13. Carefully transfer the supernatant to a new tube.

Chromatin immunoprecipitation

1. Add 300µl 1X ChIP buffer to cross-linked chromatin lysate from step 13 above.
2. To setup IP, take 200ul of lysate and add 5ug antibody of interests. Take another 200ul of lysate and add 5ug of matching rabbit or mouse IgG as negative IP control. Incubate 3 hours to overnight at 4°C with rotation. *Store remaining lysate at -80°C for future usage and for total input control and PCR positive template.*
3. Wash 10µl Protein A/G Magnetic beads with 1ml of 1X ChIP buffer in a fresh tube.
4. Transfer 200µl sample-antibody IP mixture to the tube with magnetic beads and incubate at 4°C for 1 hour.
5. Transfer the bead mixture to another **fresh** tube. This step helps reduce background.
6. Add 1 ml 1X ChIP buffer, mix well and place the tube on a magnetic stand for 30 seconds

7. Discard the buffer.
8. Repeat steps 6 –7 one more time.
9. Wash the beads by adding 1ml 1X high salt buffer, mix well and place the tube on a magnetic stand for 30 seconds.
10. Discard the buffer.
11. Wash the beads by adding 1ml of TE buffer, mix well and transfer the entire solution to a fresh tube.
12. Place the new tube on a magnetic stand for 30 seconds and discard the buffer.
13. Resuspend (by vortex) 10% chelex-100 and add 100ul of 10% chelex-100 slurry.
14. Transfer everything to a **fresh** 500ul tube and mix well for 10 seconds.
15. Boil for 10 minutes on a heat block.
16. Centrifuge for 1 minute at 10,000 rpm.
17. Carefully transfer thesupernatant to a new tube. *NOTE: Do not touch the beads as this may increase background.*
18. Perform PCR with supernatant.