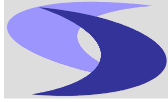


Signosis
Innovative Plate Assay Solutions

Snapshot MicroChIP assay Kit

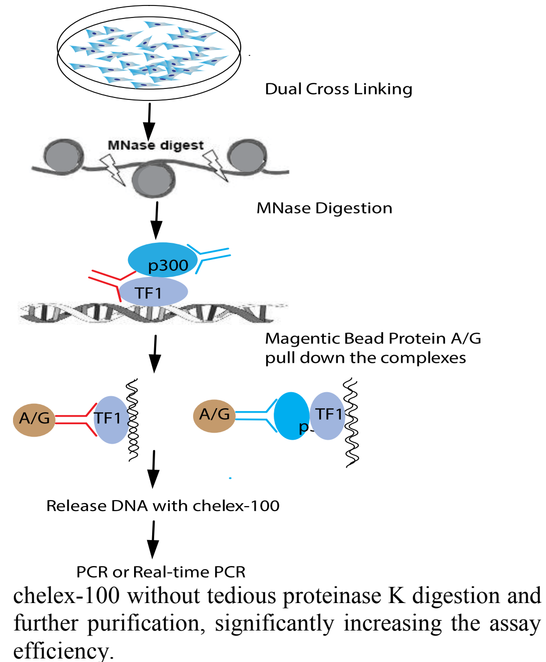
Catalog Number CA-0002 **(For Research Use Only)**

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 great improvements, Signosis developed MicroChIP assay, which can easily detect protein – promoter interaction in 500-10,000 cells. All of steps are optimized and performed in one single tube.

.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. The greatest improvement in Signosis' µChIP assay is that the staring material can be reduced from 10^6 to 500-10,000 cells and all of steps are performed in one tube. Additinal advantages 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)
- 2XCross linker buffer
- 1X Cell lysis buffer
- 1 X Nuclei Cleanup solution
- 1X MNase reaction buffer
- MNase
- 2X ChIP assay buffer
- 10XEDTA
- Protein A/G Magnetic beads
- 1X high salt wash buffer
- TE buffer
- 10% Chelex-100 solution
- 200xProteinase inhibitor
- 200xDTT

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 500-10,000 cells (a single well from 96 well plates) for each reaction This number of cells generate one chromatin ppreparation that can be used for one immunoprecipitation.
- To make crosslinker solution, dissolve 1mg of crosslinker in 20µl DMSO, and add to 3ml 1X Crosslinker buffer and mix immediately to avoid aggregation. 3ml crosslinker can be used for 30 reactions/wells (100ul each well) 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 96-well plate; for suspension cells, the cells need to be collected first and the cross-linking step can be performed in a a PCR tube.

1. Wash the cells two times with cold 1X PBS.
2. Add 100ul crosslinker solution to the cells and incubate for 20 minutes at room tempateraturе with gentle rocking.
3. Discard the crosslinker solution and wash 3 times with cold 1X PBS.
4. Add 100ul 1% Formaldehyde diluted in 1X Crosslinker buffer and incubate for 10 minutes at at room tempateraturе with gentle rocking.
5. To stop crosslinking reaction, add 12.5µl of 1M glycine and incubate for 10 minutes with gentle rocking at at room tempateraturе.
6. Remove the solution and wash the cells with cold 1X PBS three times. For suspension cells, the cells in PCR tube can be washed by pelleting up with centrifugation and resuspension. 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 and digest nucleosome, add 50ul of 1X MNase reaction buffer with gentle rocking for 10 mintues on ice.
2. Pippet up and down several times to detach the adherent from the bottom of the plate. Suspension cells still remain in the same PCR tube.
3. Add 1ul of MNase in the well (adhere cells) or in the tube (suspension cells) and incubate for 30 minutes at room temperature.
4. Stop the digestion by adding 10µl 10X EDTA with gently mixing.
5. Incubate the tube on ice for 5 minutes.
6. Add 50ul 2 X ChIP Assay buffer and mix well by gently pipetteing up and down. And incubate on ice for 10 minutes.
7. Briefly sonicate the sample for 10 seconds on ice with 10-12 kHz setting or similar seting.
8. Incubate the sample for 5 seconds on ice.
9. Repeat steps 7-8 two more times. This step will help to totally lyse the cells.

Chromatin immunoprecipitation.

1. To setup IP, transfer cell lysate (adhere cells) in a PCR tube; the suspension cells remain in the same tube.
2. Take 10ul of cell lysate for total input control (store at -80°C). Add 500ng antibody of interests to the remaining cell lysate (~100ul). Or 500ng of matching rabbit or mouse IgG as negative control. Incubate 2 hours to overnight at 4°C with rotation.
3. Wash 4µl 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.
4. Transfer 100µl sample-antibody IP mixture from Step1 to the tube with magnetic beads and incubate at 4°C for 1 hour with genetly shaking.
5. Briefly centrifuging, and then place on the magnetic stand for 30 seconds and discard the solution.
6. Add 150ul 1X ChIP Assay buffer, mix well by inverting and tapping the tube and place the tube on a magnetic stand for 30 seconds and discard the buffer.
7. Repeat steps 6 –7 one more time.
8. Wash the beads by adding 100ul 1X high salt buffer, mix well and placing the tube on a magnetic stand for 30 seconds and discarding the buffer.
9. Wash the beads by adding 100ul of TE buffer, mix well. Place the tube on a

magnetic stand for 30 seconds and discard the buffer.

10. Resuspend 10% chelex-100 resin well and transfer 25ul using a wide orifice tip into the tube and mix well with magnetic beads.
11. 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
12. Briefly centrifuge the tubes or place the tube on magnetic stand and let chelex-100 resin settled.
13. Carefully transfer thesupernatant to a new tube. *NOTE: Do not take the beads and resin in.*
14. Perform PCR with supernatant. Or conduct Next generation ChIP sequencing.

Signosis, Inc. • 1700 Wyatt Drive Suite 10-12 • Santa Clara, CA 95054 • Tel 408 747 0771 • Fax 408 864 2182