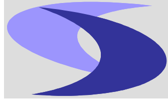


Signosis
Innovative Plate Assay Solutions

p53 Microplate-Based Snapshot ChIP Assay Kit

Catalog Number CP-0110 **(For Research Use Only)**

Introduction

p53 functions as a tumor suppressor primarily through its transcriptional regulation of a number of target genes, which encode proteins and miRNAs important for executing the p53 response to a variety of stress signals. This transcriptional activity is exerted via the direct binding of p53 to the canonicalp53 responsive DNA elements of its target promoters. Thus, monitoring the binding of p53 to its target gene promoters becomes one critical measurement of p53 transcriptional functions or specific target genes.

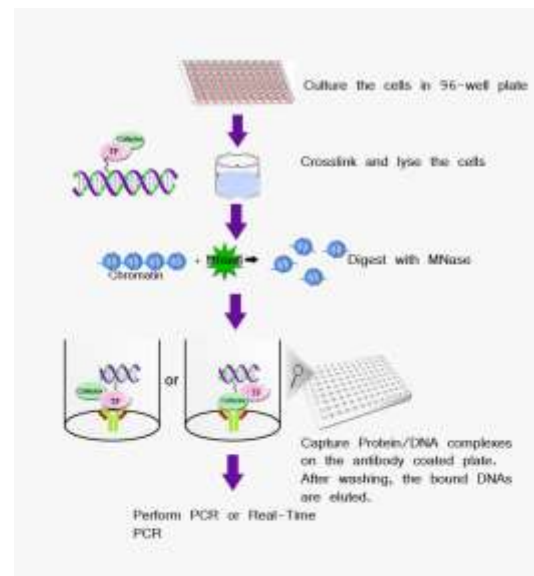
The chromatin immunoprecipitation (ChIP) assay is a major tool in the study of genomic processes and gene regulation in vivo. It is widely known control gene expression involves multiple proteins and great number of their modifications. ChIP assay is conventionally performed in test tubes one at a time, which limits the ability to gain comprehensive picture of the complex genomic events. To simplify the assay in high throughput method, Signosis has developed a 96-well plate based ChIP assay, where all of steps from IP to DNA elution are handled in microplate wells without sample transfers. This assay is an ideal tool for studying up to 96 protein-DNA bindings in one sample or one protein-DNA binding in up to 96 samples, or multiple protein-DNA bindings in multiple samples/treatments.

Principle of the assay

The assay utilizes surface-immobilized antibodies in a 96-well plate. The entire procedure from chromatin precipitation to PCR-ready DNA is performed on the same wells without sample transfers. The microplate is first blocked and coated with protein A&G, The plate is then coated with anti-p53 antibodies. The cells in 96-well culture plate or any size of dishes are cross-linked. After chromatin fragmentation, the cell lysates are transferred and incubated on the antibody-coated plate to form antibody-protein-DNA complex. After several wash steps, the bound DNAs are subsequently eluted. The eluted DNAs can be used for PCR analysis or next generation sequencing. The microplate-based ChIP assay has several important advantages over the tube-based assay: simple sample handling, high throughput, improved sensitivity and reproducibility.

Materials provided with the kit

- p53-antibody coated capture plate
- 1X MNase reaction buffer
- MNase
- 2X ChIP assay buffer
- 10X EDTA
- 1X High salt wash buffer
- TE buffer
- Elution buffer
- p53 control primers



Material required but not provided

- 1X PBS
- 37% Formaldehyde
- 1M Glycine
- DMSO
- Sonicator
- Rocker platform
- Incubator

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Reagent preparation before starting experiment

- Culture and treat 1-2x10^4 cells/well in a 96 well plate or any size of culture dishes depending on experimental design. 1-2x 10^4 cells/well can be used for one IP. 2-5x10^6 cells/10cm dish can be for 100 IPs
- Prior to use, prepare the working buffers by following the instructions on the product insert.

Assay procedure

Cross-linking, Lysis of the cells and fragmentation

Cells cultured in 96-well plate

1. Add 2.7ul of 37% formaldehyde into 100ul cell culture medium. Or replace the entire medium with 100ul 1% formaldehyde containing medium.
2. Incubate for 10 minutes at room temperature with gentle rocking.
3. Add 3.25ul of 1M glycine per well to stop crosslinking reaction, and incubate for 10 minutes with gentle rocking at room temperature.
4. 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.
5. To lyse cells, add 50ul of 1X MNase reaction buffer with gentle rocking for 10 minutes on ice.
6. Detach the cells by pipetting up and down several times and add 1ul MNase enzyme and incubate for 30 minutes at room temperature with gently shaking.
7. Add 5ul of 10X EDTA to stop the reaction and add 50ul 2X ChIP assay buffer pipetting up and down several times.
8. Briefly sonicate the samples for 5 seconds on ice with 10-12 kHz setting or similar setting by putting the probe in the middle of the well. Repeat two more times.

Cells cultured in 10cm^2 dish

1. Add 270ul of 37% formaldehyde in 10ml cell culture medium and incubate for 10 minutes.
2. Add 325µl of 1M glycine to stop the crosslinking reaction.
3. Remove the solution and wash the cells with cold 1X PBS three times. Remove as much PBS as possible after the last wash.
4. Add 1ml 1X MNase reaction buffer and incubate on Ice for 10 minutes.
5. Scrap cells off the plate and collect in a microcentrifuge tube, and centrifuge at 3000 rpm for 5 min to pellet nuclei.
6. Discard the supernatant and well resuspend the pellet in 400ul 1X MNase reaction buffer by gently pipetting up and down until this nuclei suspension looks like a milky opalescent luster.
   Optional for further cleanup for the cell number over 10^7
   a. Add 400ul 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.
   b. Centrifuge for 5 minutes at 3000 rpm to pellet nuclei through.
   c. Remove the buffer and resuspend the pellet in 400µl 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 evenly resuspend the nuclei in solution)
7. Add 6ul of MNase, 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 40µl 10X EDTA
9. Add 400ul 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. Add 100ul blocking buffer in each well of protein A& G coated capture plate and add 200ng antibody in blocking buffer, and mix well by gently pipetting up and down.
2. Incubate for 1 hour at room temperature to allow antibody binding on the plate. Discard the solution and wash twice with 1X PBS.
3. Add 100ul chromatin prep from each well of 96-well cell culture plate to one well of antibody coated capture plate. Or Add 8ul chromatin prep from a 10cm^2 plate in 100ul 1 x ChIP Assay Buffer to one well of antibody coated capture plate.

4. Incubate for 1-2 hours at 4ºC.
5. Invert the plate over an appropriate container and expel the contents forcibly. Wash the plate by adding 200µl of 1x ChIP Assay wash buffer. Repeat the washing process one more time for a total of two washes. Completely remove liquid after each wash by firmly tapping the plate against a pile of clean paper towels.
6. Wash wells with 200ul 1X High salt buffer once. Completely remove liquid by firmly tapping the plate against a pile of clean paper towels.
7. Wash with 200ul TE buffer once. Completely remove liquid by firmly tapping the plate against a pile of clean paper towels.
8. Add 100ul elution buffer, and incubate at 55°C for 20 minutes.
9. The eluted DNA is ready for downstream analysis.