Tribo™ Chromatin Immunoprecipitation (ChIP) Assay (Catalog# TBS8050)

DESCRIPTION

Chromatin Immunoprecipitation (ChIP) is a powerful tool for analysis of interaction of proteins associated with chromosomal DNA. These proteins can be histone subunits and post-translational modification, or other chromatin associated proteins such as transcription factors, chromatin regulators. Additionally, ChIP can be used to identify regions of the genome associated with these proteins, or conversely, to identify proteins associated with a particular region of the genome.

Tribo™ ChIP Assay Kit is designed to use analysis of 25reactions for chromatin modification in tissue or cell.

KIT CONTENTS

ChIP Kit:

Protein A/G Magnetic Beads: 500 µL
Cell Lysis Buffer: 15 mL
Nuclear Lysis Buffer 15 mL
ChIP Dilution Buffer: 25 mL
Wash Buffer 1 (Low Salt): 25 mL
Wash Buffer 2 (High Salt): 25 mL
Wash Buffer 3 (LiCl): 25 mL
Wash Buffer 4 (TE): 25 mL
ChIP Elution Buffer: 1.5 mL
10x Glycine, 5mL
Proteinase K (20 mg/mL): 0.5 mL
Protease inhibitor Cocktail II (PI): 0.2 mL (-20 ˚C)
Normal Mouse IgG

Chromatin Purification Kit

Binding Buffer A: 12.5 mL
Washing Buffer B: 12.5 mL
Elution Buffer C: 2.5 mL
Spin Column: 25

OTHER COMPONENTS REQUIRED BUT NOT INCLUDED AS PART OF KIT

Antibody for ChIP
1 x PBS

APPLICATIONS

Chromatin modification for tissue or cell.

STORAGE CONDITIONS

The Kit stored at4˚C, and stable for 6 months from date of shipment.

PROTOCOL

A: Tissue Process

1. Quickly mince tissue using razor blades in 1.0 mL cold-PBS-PI in 10-cm culture dish on ice. Transfer the minced tissue to a new 2 ml microtube.
2. Mash the tissue as much as possible with the 1-ml pipette.
3. Simply spin the sample at 4˚C and discard the supernatant and keep the pellet in the tube.

B: X-linking the chromatin

4. Add 1ml 1% formaldehyde in PBS-PI in each tube and resuspend the pellet. Incubate the sample for 10 min at room temperature. (1% Formaldehyde-PI: add 135µL of 37% formaldehyde to 5 mL of 1xPBS-PI).
5. Stop x-linking by adding 100 µL of 10 x glycine to the sample (10 times dilution of 1.25M glycine to final concentration of 0.125M). Incubate for 5 min at room temperature.
6. Simply spin the linked sample and pour off the supernatant and keep the pellet.
7. Wash 2xtimes with cold PBS-PI (1 ml). (The tissue may be snap frozen at this stage in store -80˚C.)

C: Lysis and Sonication

8. Add 0.5 ml of Cell Lysis Buffer with PI to each pellet. Resuspend by mild pulses on the vortex mixer. Incubate on ice for 15 min; vortex every 5 min.
9. Simply spin at 2000x g, 4˚C for 2min
10. Remove the supernatant and resuspend the cell in 0.5 mL nuclei lysis buffer.
11. Homogenize the sample with glass Dounce Homogenizer (1ml) on ice for 10 strokes. Incubate the tube on ice for at least 10 min.
12. Sonicate the sample on ice to shear DNA to an average fragment size of 200~ 1000 bp. This will need optimizing as different equipment and tissue.
13. Centrifuge the sonicated chromatin at 10,000xg at 4 ˚C for 10 min(Do not exceed 15000 x g to prevent loss of chromatin). The supernatant is chromatin of the sample.
14. Remove supernatant to fresh microfuge tubes in 50- to 200 µL aliquots. Each 50 uL aliquot contains 1x 10^6 cell equivalents of lysate which is enough for one immunoprecipitation. Proceed to the next step or store at -80˚C for 2 months.

15. To determine the DNA concentration, transfer 5 µl of the purified DNA into a tube containing 995 µl TE to give a 200-fold dilution and read the OD260. The concentration of DNA in µg/mL is OD260 x10,000. This used to calculate the DNA concentration of the chromatin preparation.
16. Perform agarose gel analysis of sonication (optional) Remove a 10-25 µL aliquote, and incubate for 10 min at 95 C. Add 1 µL Proteinase K and incubate at 62C for 2 hours with shaking. Load 10 µL or 20 µL on a 2% agarose gen with a 100-bp DNA marker.

D. Immunoprecipitation

17. Prepare enough Dilution Buffer containing protease inhibitors for the number of desired immunoprecipitations and store on ice. Add dilution buffer to each sample as shown bwelow:

Sample Vol.	Dilution Buffer Vol.	PI Vol.
50 µL	450 µL	2.25 µL
100 µL	900 µL	4.5 µL

18. ChIP reaction includes the negative control (Normal Mouse IgG) and the antibody of interest (user supplied). It is recommended that the negative control IgG be from the same species as the antibody of interest.
19. Remove 5 uL of the supernatant as Input and save at 4˚C until Section E (Reverse Cross-links of Protein/DAN Complexes to Free DNA).
20. Add target antibody (5 µg in 500 µL total volume) to each reaction tube.
21. For the negative control, Normal Mouse IgG, add 1.0 ug of antibody per tube.
22. Add 20 uL of fully resuspended protein A/G magnetic Beads to each reaction tube (Magnetic bead Slurry is well mixed before removing appropriate volume for IP, as magnetic bead will settle on the bottom of the tube over a short period of time).
23. Incubate for 1 hour to overnight at 4˚C with rotation.
24. Pellet Protein A/G magnetic beads with the magnetic separator or spin at 1500 rpm for 30 seconds. Remove the supernatant completely.
25. Wash the protein A/G bead-antibody/ chromatin complex by resuspending the beads in 0.5 mL each of the cold buffers in the order listed below and incubate for 3-5 min on a rotating platform between each wash followed by magnetic separation and careful removal of the supernatant fraction:
    Low Salt Wash Buffer 1, one wash.
    High Salt Wash Buffer 2, one wash
    LiCl Wash Buffer 3, one wash.
    TE Buffer 4, one wash

E. Isolate DNA

27. Warm the ChIP Elution Buffer to room temperature to ensure the SDS is in solution before proceeding. Prepare the final elution buffer for all samples including input (prepared in Section D, Step 18). For each tube, prepare elution buffer as follows:

ChIP Elution Buffer	100 µL
Proteinase K	1 µL

28. Incubate at 62 ˚C for 2 hours with shaking. Then, Incubate at 95 ˚C for 10 min. Cool the samples down to room temperature.
29. Separate beads using a magnet separation device or spin at 2000 rpm for 30 seconds to drop down the beads to bottom of the tube. Carefully transfer the supernatant to a new tube.

F. DNA Purification Using Spin Columns

30. Add 0.5 mL of Bind Reagent A to each 100uL DNA sample tube, and mix (5 volume s of Bind Reagent A is used for every 1 volume of sample). A precipitate may be observed. This will not interfere with this procedure.
31. Transfer the sample/Bind Reagent A mixture to the spin column.
32. Spin at 10,000rpm for 30 seconds, discard the pass-through liquid.
33. Add 700 ul of Wash Reagent B to the column, and spin again as before. Discard the pass-through. Put the column back to the same tube, and spin at 10,000 rpm for 1 min.
34. Put the column into a clean Collection Tube.
35. Add 50 uL Elution Buffer C directly onto the center of the column. Spin at 10,000 rpm for 30 seconds.
36. Remove and discard the column. The eluate is purified DNA. It can be analyzed immediately or stored frozen at -20 ˚C. The DNA can be used for PCR analysis.

RELATED PRODUCTS

2x qPCR Hot Start Super Mix (Catalog# TBS4001)

2x PCR Hot Start Master Mix Catalog# TBS4002)

This product is for *in vitro* research use only and is not intended for use in humans or animals in therapeutic and diagnosis purpose.