



Chromatin Immunoprecipitation (ChIPs) Protocol (Mirmira Lab)

Updated 12/3/02

Reagents:

•ChIP sonication Buffer (1% Triton X-100, 0.1% Deoxycholate, 50 mM Tris 8.1, 150 mM NaCl, 5 mM EDTA):

10 ml 10 % Triton X-100
1 ml 10 % Deoxycholate
5 ml 1 M Tris-HCl pH 8.1
1 ml 0.5 M EDTA
3 ml 5 M NaCl
80 ml Water

Just before use, add 10 μ l Aprotinin, 10 μ l Leupeptin, and 5 μ l PMSF to each 10 ml.

•High Salt Wash Buffer (1 % Triton X-100, 0.1 % Deoxycholate, 50 mM Tris-8.1, 500 mM NaCl, 5 mM EDTA)

10 ml 10 % Triton X-100
1 ml 10 % Deoxycholate
5 ml 1 M Tris-HCl, pH 8.1
1 ml 0.5 M EDTA
10 ml 5 M NaCl
73 ml Water

•LiCl Immune Complex Wash Buffer

25 ml 1 M LiCl
5 ml 10 % IGEPAL
5 ml 10 % Deoxycholate
1 ml 1 M Tris-HCl, pH 8.1
200 μ l 0.5 M EDTA
64 ml Water

•Protease inhibitors (add 10 μ l of each to 10 ml of PBS or sonication buffer)

Leupeptin 2 mg/ml in water
Aprotinin 2 mg/ml in water
PMSF 0.2 M



- 5 M NaCl
- 1X TE Buffer (10mM Tris, 8.1, 1 mM EDTA)
- 0.5 M EDTA
- 1 M Tris-HCl, pH 6.8
- Protein A/G-Agarose
- Proteinase K (19 mg/ml, Boehringer Mannheim # 1964372)
- 10X proteinase K buffer
- Elution Buffer (1% SDS, 0.1 M NaHCO₃, 0.01 mg/ml Herring sperm DNA)
- 10 mg/ml Herring Sperm DNA
- 37% Formaldehyde (ACS reagent grade)
- 1.25 M glycine

Protocol: (Generalized for all cell types)

For all the following steps, use the pipets that are specifically designated for ChIP use only and the filter pipette tips.

1. To each 10 cm dish of cells, wash plate once with 10ml of PBS, then add 10 ml of Fresh PBS and add 270 μ l of 37 % formaldehyde, swirl gently to mix, and place at room temp 10 min.
2. At the end of the incubation, add 1 ml of 1.25 M glycine, swirl to mix.
3. Aspirate medium
4. wash plate with 10 ml cold PBS x 2. Aspirate PBS completely after the second wash.
5. Add 500 μ l of cold PBS + protease inhibitors and scrape cells, collect in a 1.5 ml centrifuge tube. At this point you should pool three plates worth of cells together in the same tube (I suggest using a 2 ml eppendorf tube for this purpose).
6. Centrifuge at 2,000 rpm for 2 min at 4°C.
7. Remove and discard PBS
8. Add 600 μ l of ChIP sonication buffer + protease inhibitors, and resuspend pellet (you can vortex vigorously at this point). Transfer the suspension to a 1.5 ml eppendorf tube.



9. Place on ice for 10 min.
10. Sonicate at a setting of 10 or 11 for 15 (possibly fewer, but 15 will ensure sufficient shearing of the DNA) pulses, 5 seconds per pulse (placing the cells on an ice-water bath between pulses). To eliminate foaming, place the tip of the horn near the bottom of the tube while touching the side with the horn. *USE THE PLAIN WHITE TUBES.*
11. Centrifuge at maximal setting at 4°C for 10-15 min.
12. Remove the supernatant into a fresh tube. This is the Whole Cell Extract (WCE), and can be stored at -80°C at this point, if desired.
13. Add a sufficient amount of ChIP buffer to perform the immunoprecipitations. A final volume between 1-1.5 ml is usually good. Add protease inhibitors to this (about 2 µl of each PMSF, aprotinin, and leupeptin) and place on ice.
14. Add the luciferase plasmid before splitting the samples. One microliter of stock plasmid (about a couple of micrograms) is good. Also add 10 µl of 100X BSA. Mix well.
15. Split the samples into (+) and (-) antibody samples of equal volume, making sure you choose an amount that will allow you to withhold 10 % of the IP sample volume for your “10% input” samples that you need later for PCR, as well as having a little leftover sample in case you want to examine them later. For example, if you have 1 ml after step 13, split it into a 400 µl (+) sample, a 400 µl (-) sample, a 40 µl input sample, with 160 µl leftover.
16. Label and freeze the 10 % Input samples and leftovers.
17. To the (+) antibody sample, add 1 µl of antibody. This amount may vary according to the antibody used and the size of your sample. To the (-) antibody sample, add nothing.
18. Place the samples on a nutator in the cold room, and rotate **overnight**. This step could also be done only for 2hrs in the cold room.
19. Resuspend Protein A/G-agarose so that it forms a uniform suspension. Using a pipet tip with the end clipped off, add 40 µl of this suspension to each immunoprecipitation. Resuspend the protein A/G-agarose each time before adding to the next sample, as it settles quickly.



20. Add 2 μ l of 10 mg/ml solution of herring sperm DNA
21. Place back on the nutator at 4°C for 1-2 h.
22. Centrifuge the samples at 4°C for 1 min at 2,500 rpm.
23. Carefully remove the supernatant using a P-1000 and place it in a tube and label it “sample X-sup.” Place this at –20°C in case you need it later.
24. Add 1 ml of cold CHIP buffer (no protease inhibitors), invert the sample to resuspend the resin, and centrifuge for 1 min. at 2,500 rpm.
25. Remove and discard the supernatant.
26. Wash 2X in cold PBS, spinning as above and discarding the supernatants. *Note: wash buffer may vary according to conditions, antibody used. See alternate wash buffers at top of protocol.*
27. Add 250 μ l of Elution buffer to the resin, and place on a nutator at room temp. for 15-20 min.
28. Centrifuge at top speed 60s to pellet the resin, remove the supernatant to a fresh tube.
29. Repeat the elution step (step 16), except that it is recommended to place tubes in a 100°C heat block for 60s before placing on the nutator.
30. Spin as in step 29 and combine with the supernatants from step 29. (you can now discard the tube containing the resin pellet)
31. At this time, add 500 μ l of elution buffer to the “10 % input samples” from step 16. Process them along with your other samples from here on.
32. Add 20 μ l of 5 M NaCl to each sample, vortex to mix, and place in a 65°C bath for 3-4 h.
33. Add 1 ml of room temperature-ethanol to each sample place at –20°C **overnight**.
34. Next day, spin the samples at top speed at 4°C for 15-20 min. to pellet the precipitated



protein/DNA. Be sure to pre-chill the centrifuge.

35. Aspirate off the supernatant, add 1 ml of ice cold 70 % ethanol, spin again at 4°C for 5 min.
36. Aspirate off the sup, allow to air dry for 5-10 min.
37. Dissolve the pellet in 100 µl of TE.
38. Add 11 µl of 10X Proteinase K buffer, and 1 µl of a 19 mg/ml proteinase K solution.
39. incubate at 55°C for 1 h.
40. Add 390 µl TE
41. Extract with 500 µl phenol : CHCl₃ : isoamylalcohol
 - a. Add the stuff
 - b. Vortex high speed 1 min
 - c. Spin high speed 1 min
42. Remove top (aqueous phase) and put into new tube.
43. Add 44 µl 3 M NaOAc and 1 ml EtOH
44. Place at -20°C **overnight**
45. Spin sample at high speed 4°C for 5 min.
46. Aspirate sup. Add 1 ml ice cold 70 % EtOH. Spin again high speed 4°C for 5 min.
47. Aspirate sup, air dry pellet about 20 min.
48. Resuspend pellets in 100 µl TE (vortex).
49. Ready for PCR.
50. If samples, especially the inputs, give strange looking or flat curves on the I-cycler, try adding 390 µl TE to the samples and repeating steps 44-50.