



Chromatin Immunoprecipitation (ChIPs) Protocol (Farnham Lab)

This protocol is based upon protocols from Mark Biggin, Dave Allis and Richard Treisman plus a fair amount of trial and error. We have successfully used this protocol with NIH 3T3, Friend, HeLa, Raji and CHO cells. However, you may have to optimize conditions for your specific cell type.

Day 1

1. Add formaldehyde directly to tissue culture media to a final concentration of 1 %. We generally use 2×10^7 cells per antibody per timepoint. Fewer cells can be used but usually results in a lower signal to noise ratio. Incubate adherent cells on a shaking platform and suspension cells on a stir plate for 10 minutes at room temperature. Crosslinking for longer periods of time (>30 minutes) tends to cause cells to form into a giant crosslinked aggregate that can not be efficiently sonicated.
2. Stop the crosslinking reaction by adding glycine to a final concentration of 0.125 M. Continue to rock or spin at room temp for 5 minutes.
3. For adherent cells, pour off media and rinse plates twice with cold 1X PBS. For suspension cells, centrifuge and wash cell pellet twice with cold 1X PBS.
4. Add an appropriate volume (we use 1 ml per 100 cm² dish) of 1X PBS or a 20 % 1X trypsin/EDTA (tissue culture grade) solution in 1X PBS. Incubate at 37°C for 10 minutes if using trypsin (we have found this step useful for cells, which are difficult to swell. Thus, for cell types that are easily swelled, this step may not be necessary).
5. Following addition of trypsin or PBS, scrape adherent cells from dishes. If you have used trypsin, inactivate the trypsin by adding a small amount of serum. Centrifuge scraped adherent or suspension cells and wash pellet once with 1X PBS plus PMSF (10 µl per ml).
6. Resuspend cell pellet in cell lysis buffer plus the protease inhibitors PMSF (10 µl per ml), aprotinin (1 µl per ml) and leupeptin (1 µl per ml). The final volume of cell lysis buffer should be sufficient so that there are no clumps of cells. Incubate on ice for 10 minutes. Cells can also be dounced on ice with a B dounce several times to aid in nuclei release.
7. Microfuge at 5,000 rpm for 5 minutes at 4°C to pellet the nuclei.



8. Resuspend nuclei in nuclei lysis buffer plus the same protease inhibitors as the cell lysis buffer. Incubate on ice for 10 minutes.

9. Sonicate chromatin to an average length of about 600 bp while keeping samples on ice (the time and number of pulses will vary depending on sonicator, cell type and extent of crosslinking). Microfuge at 14,000 rpm for 10 minutes at 4°C. At this point, chromatin can be snap frozen in liquid nitrogen and stored at -70°C for up to several months.

10. Carefully remove the supernatant and transfer to a new tube. Preclear chromatin by adding blocked Staph A cells. Use 10-15 µl of preblocked Staph A cells for every 1×10^7 cells that you started with.

11. Incubate on a rotating platform at 4°C for 15 minutes, no longer. Microfuge at 14,000 rpm for 5 minutes.

12. Transfer supernatant to a clean tube and divide equally among your samples. Be sure to include a "no antibody" sample. We also include a "mock" samples which contains 1X dialysis buffer instead of chromatin (no antibody and mock are critical to control for nonspecific interactions and DNA contamination of IPs and wash solutions; the final output of this experiment is analyzed by PCR). Adjust the final volume of each sample with IP dilution buffer plus protease inhibitors if necessary. Samples volumes should be between 200 and 500 µl. Add 1 µg of antibody to each sample.

13. Incubate on the rotating platform at 4°C for at least 3 hours. Overnight is fine. If you are using monoclonal antibodies, add 1 µg of an appropriate secondary antibody and incubate for an additional 1 hour.

Day 2

14. Add 10 µl of blocked Staph A cells to each sample. Incubate on the rotating platform at room temp for 15 minutes, no longer.

15. Microfuge samples. Save the supernatant from the "no antibody" sample as "total input chromatin".

16. Wash pellets twice with 1.4 ml of 1X dialysis buffer (**if you are using a monoclonal antibody, omit the sarkosyl**) and four times with 1.4 ml of IP buffer (**pH 8.0 for monoclonal antibodies**).



DNA in water or TE and analyze by PCR. We generally resuspend in IP's in 30 μ l and then dilute the "total" sample an additional 300 fold and use 2-3 μ l for each PCR reaction.

Solutions

Cell Lysis buffer

5 mM PIPES pH 8.0

85 mM KCL

0.5 % NP40

protease inhibitors

Nuclei Lysis buffer

50 mM Tris-Cl pH 8.1

10 mM EDTA

1 % SDS

protease inhibitors

IP Dilution buffer

0.01 % SDS

1.1 % Triton X 100

1.2 mM EDTA

16.7 mM Tris-Cl pH 8.1

167 mM NaCl

1X Dialysis buffer

2 mM EDTA

50 mM TrisCl pH 8.0

0.2 % Sarkosyl (omit for monoclonal antibodies)

IP Wash buffer

100 mM Tris-Cl pH 9.0 (8.0 for monoclonal antibodies)

500 mM LiCl

1 % NP40

1 % deoxycholic acid

**Elution buffer**

50 mM NaHCO₃

1 % SDS

5X PK buffer

50 mM Tris-Cl pH 7.5

25 mM EDTA

1.25% SDS

Protease Inhibitors

100 mM PMSF in ethanol, use at 1:100

10 mg per ml aprotinin in 0.01 M HEPES pH 8.0, use at 1:1,000

10 mg per ml leupeptin in water, use at 1:1,000

Staph A Cells

Resuspend 1 g of lyophilized Staph A cells (Boehringer Mannheim) in 10 ml of 1X dialysis buffer. Centrifuge at 10,000 rpm for 5 minutes at 4°C. Repeat. Resuspend in 3 ml of 1X PBS plus 3 % SDS and 10 % betaME. Boil for 30 minutes. Centrifuge at 10,000 rpm for 5 minutes. Wash in 1X dialysis buffer and centrifuge at 10,000 rpm for 5 minutes. Repeat. Resuspend in 4 ml of 1X dialysis buffer. Divide into 100 µl aliquots, snap freeze and store in liquid nitrogen.

To block Staph A cells

Thaw 1 tube (100 µl) of cells for approximately every 10⁸ cells that you begin with. Add 10 µl of herring sperm DNA (10 mg/ml) and 10 µl of BSA (10 mg/ml) to each tube of Staph A cells. Incubate on the rotating platform at 4°C for at least 3 hours, overnight is fine. Before using, microfuge for 3 minutes. Remove supernatant and wash pellet twice in 1X dialysis buffer. Resuspend cells in a volume of 1X dialysis equal to the original starting volume.