



The Power to Quantify

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# ChIP Assay Protocol



- Wash cells twice with PBS at room temperature, resuspending to approximately  $5 \times 10^5$  cells/ml (approximately  $2 \times 10^7$  cells total). Add formaldehyde to a final concentration of 1% and incubate at room temperature for 10 minutes.
- Terminate cross-linking reactions by adding glycine to a final concentration of 0.125 M.
- Pellet cells (2,000 RPM, 5 minutes) and wash once with ice cold PBS.
- Resuspend cells in 6 ml Lysis Buffer ([sc-45000](#)) by mixing gently.
- Collect crude nuclear extract by microcentrifugation at 2,000 RPM, 5 minutes.
- Wash again with PBS. Pellet may be frozen or processing may be continued as follows:
  - a) Resuspend pellet in  $\sim 1.9$  ml Lysis Buffer High Salt ([sc-45001](#)) and transfer to 2 ml microcentrifuge tube for the sonication step.
  - b) Sonication conditions should be optimized since results may vary using different sonifiers. The following conditions were established by using a Sonics VC130 with a 3 mm tip probe.
  - c) Sonicate on ice at power output setting = 5-6, continuous mode, 4 times at 30 second intervals.
  - d) Centrifuge extract for 15 minutes, 10,000 rpm at  $4^\circ\text{C}$  and save supernatant (chromatin).
  - e) Determine protein concentration of supernatant.
  - f) For the IP step we recommend using 100-500  $\mu\text{g}$  protein and 0.1-1  $\mu\text{l}$  TransCruz reagent (0.2-2  $\mu\text{g}$ ).

**NOTE:** Investigators may wish to consider using the primary antibody conjugated to sepharose or magnetic beads as an alternative to using secondary immunoprecipitation reagents (e.g., Protein A-Agarose) as described here. Combining primary antibodies directed to different epitopes of the same protein may be advantageous in some cases.

- Preclear the chromatin solution by adding 50  $\mu\text{l}$  Protein A/G PLUS Agarose ([sc-2003](#)) and incubate for 30 minutes at  $4^\circ\text{C}$ . Centrifuge at full speed for 5 minutes at  $4^\circ\text{C}$ .
- Add primary antibody to the supernatant and incubate overnight at  $4^\circ\text{C}$ .
- Add 50  $\mu\text{l}$  Protein A/G PLUS-Agarose ([sc-2003](#)) and incubate for 2 hrs at  $4^\circ\text{C}$ .
- Harvest beads by centrifugations at 12,000 rpm for 20 seconds and place tube in ice.
- Wash beads twice with 1 ml Lysis Buffer High Salt ([sc-45001](#)).
- Wash pellet four times with Wash Buffer ([sc-45002](#)).
- Resuspend beads in 400  $\mu\text{l}$  Elution Buffer ([sc-45003](#)).
- Reverse cross-links by incubating tube in a  $67^\circ\text{C}$  water bath, mixing occasionally over two hours. Remove beads by centrifugation and continue incubating supernatant at  $67^\circ\text{C}$  overnight.
- Centrifuge for 3 minutes at 10,000 to remove any residual beads and save supernatant.
- To isolate DNA, extract supernatant once with 500  $\mu\text{l}$  phenol/chloroform/isoamyl alcohol (25:24:1), vortex thoroughly and separate phases by centrifuging tube for 3 minutes at 14,000 rpm.
- Save the aqueous phase, back extract the organic phase once with 100  $\mu\text{l}$  10 mM Tris, 1 mM EDTA, pH 8.1 (TE) and pool aqueous phases.
- Extract pooled aqueous phase with 600  $\mu\text{l}$  chloroform/isoamyl alcohol.
- DNA may be concentrated by using commercially available kits.