

ChIP from tissues with the LowCell# ChIP kit

Diagenode - June 2008 - Additional information

In theory, you could use **30 mg** of tissue per antibody per ChIP.

You will start by optimizing conditions for the type of tissue you work with.

The exact amount of tissue to start with depends on the relative abundance of the protein of interest in the tissue, on how good the antibody is (ChIP grade, ...) and also on how efficient the crosslinking is, ... and so on.

In another words, conditions must be optimized with each sample and conditions.

At the end of this protocol for chromatin preparation from tissues, it is possible to continue in LowCell# ChIP (the kit can be plugged, as described below).

In that case, resuspend nuclei in 130 μ l of Buffer B (RT) (LowCell# ChIP kit reagent) and proceed to "Bioruptor shearing". Afterwards, dilute the sheared chromatin with 870 μ l of Buffer A as described in the manual from the LowCell# ChIP kit and perform ChIP.

1. Weight frozen or fresh tissue.
2. Chop tissue into small pieces using 2 razor blades (between 1-3 mm³).
3. Transfer tissue into a tube with a screw cap lid and add 10 ml PBS (plus protease inhibitors) per gram of tissue.
4. Add formaldehyde to a final concentration of 1% and rotate tube at room temperature (RT) for 15 minutes.
5. Stop the cross-linking reaction by adding fresh glycine to a final concentration of 0.125 M. Continue to rotate at RT for 5 minutes.
6. Centrifuge tissue samples at low speed (100x g or 707 rpm) at 4°C.
7. Aspirate the supernatant and wash the pellet once with ice-cold PBS (plus protease inhibitors). Centrifuge.
8. Resuspend tissue in 10 ml ice-cold PBS (plus protease inhibitors) per gram of starting material. Keep on ice.

Tissue disaggregation For this step, use a Medimachine from Becton Dickinson to achieve a single cell suspension. Use 2 medicones (50 μ m) per gram of tissue to process.

1. Cut a 1000 μ l pipette tip (larger hole will ease pipetting).
2. Add between 50-100 mg of tissue resuspended in 1 ml of PBS in each medicone.
3. Grind tissue for 2 minutes.
4. Collect cells from medicone by inserting an 18 g blunt needle and a 1 ml syringe. Keep them on ice.
5. Keep adding between 50-100 mg of tissue resuspended in 1 ml of PBS in each medicone until all the tissue is processed.
6. If necessary, add more PBS (plus protease inhibitors) to the tissue sample to get an homogeneous suspension.
7. Check cell suspension by microscopy.
8. Centrifuge cells at 1,000 rpm at 4°C. Estimate the cell pellet volume.

Chromatin Preparation

1. Resuspend the cell pellet in 6 x volumes of cell lysis buffer (5 mM PIPES pH 8.0, 85 mM KCL, 0.5% Igepal [add fresh]) supplemented with protease inhibitors: PMSF (10 µl/ml), aprotinin (1 µl/ml) and leupeptin (1 µl/ml). The final volume of cell lysis buffer should be large enough to avoid clumps of cells.
2. Incubate on ice for 10-15 minutes.
3. Centrifuge nuclei at 1000x g (2235 rpm) at 4°C.
4. Discard supernatant. Keep the pellet.
5. Resuspend nuclei in **130 µl of Buffer B (RT)** (LowCell# ChIP kit reagent). Vortex until resuspension.
6. Incubate for 5 minutes on ice.
7. Submit the samples to sonication to shear the chromatin using the Bioruptor™ for 12 cycles of: [30 seconds "ON", 30 seconds "OFF"] each.
8. Use the sheared chromatin directly in ChIP.
9. Add 5 µl of Protease Inhibitor mix per ml of **Buffer A** (LowCell# ChIP kit reagent). e.g. add NaBu (20 mM final) or any other inhibitor to **Buffer A**.
10. Add 870 µl of complete **Buffer A** to the 130 µl of sheared chromatin.

Note: With 1 ml of sheared chromatin (130 µl Buffer B + 870 µl Buffer A), you can do 10 ChIP assays (using 100 µl **per ChIP**). Then the starting material should be ± 10 x **30 mg** of tissue.