diagenode
Innovating Epigenetic Solutions

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Cell Fixation Protocol for Histone Low Cell amounts

Cell amount:

Standard quantities per IP:

- ChIP on **histones low cell amount**: >20.000 – 500.000 cells

The total number of cells required will depend on the experimental design of the project (e.g. number of targets to analyse). In each ChIP experiment one positive (H3K4me3) and one negative (IgG) control are included in the work flow which need to be considered as 2 additional IP reactions. These quantities are adaptable depending on sample availability.

Additional test samples need to be provided for shearing optimization (2 test samples per cell/tissue type with the same cell amount as calculated for the samples of interest) and for the AB validation (x tests samples (depending on the validation design) per cell/tissue type with the same cell mount as calculated for the samples of interest).

Please consult your Service specialist to determine the proper cell amounts for your project.

Reagents:

- DPBS (Life technologies, 14190-094)
- Trypsin-EDTA 0,05% (HyClone, SH30236.01)
- Cell culture medium, use the same medium as for culturing the cells
- Formaldehyde 37% (Sigma, F8775-25ML)
- Glycine 1.25 M (VWR, 1.04201.0250)

Notes before starting:

This protocol has been optimized for shearing of **a minimum of 20.000 cells** in 100 µl using the Diagenode's Bioruptor® and then subsequent immunoprecipitation of 20.000 cells in 200 µl. Determine the number of IP you will perform and start with fixation of a unique batch of chromatin. For example, if you would like to perform 4 ChIPs on the same chromatin, start with fixation of 100.000 cells minimum (1 extra chromatin preparation to use for the input and shearing analysis).
Due to the low amount of starting material it is critical to avoid sample loss throughout the experiment to ensure reproducible and consistent results. Avoid pipetting up and down when adding buffers to samples. It is also recommended to use low retention Eppendorf tubes at each step of the protocol to minimize sample lost. The centrifugation speed might also be adjusted depending on the cell type.
The use of an automated cell counter is also recommended to reduce variations in the amount of the starting cell number.

Protocol:

1. Prepare and harvest cells as follows: Place PBS, cell culture medium and trypsin-EDTA at room temperature (RT). If using adherent cells, discard medium to remove dead cells. Wash cells by adding 10 ml PBS. Detach cells by trypsinization. Collect cells by adding culture medium and transfer the medium with cells in a 15 ml centrifugation tube. Use culture medium containing serum (you can use the same medium as the one used for culturing the cells). Centrifuge 5 minutes at 500 x g (or higher speed depending on your cell type). Make sure that the speed used results in cell pelleting without damaging the cell membrane. Keep the cell pellet and discard the supernatant. If using suspension cells, centrifuge for 5 minutes at 500 x g (or higher speed depending on your cell type). Keep the cell pellet and discard the supernatant.
2. Resuspend the cells in cell culture medium. You should have **at least** 100 000 cells per ml of cell culture medium. Count the cells.
3. Label new 1.5 ml tube(s). Add medium to a final volume of 1 ml after the cells have been added. To determine the amount of cells to use for fixation, determine the number of immunoprecipitation you will perform and start fixation of a unique batch of chromatin (see also notes before starting).
4. Add 27 µl of 36.5% formaldehyde per 1 ml of sample (**final concentration should be ~1%**) and invert tubes immediately two to three times to ensure complete mixing.
5. Incubate for 8 minutes at room temperature to enable fixation with occasional manual agitation. Optimization of fixation time may be required depending on cell type, it could be 8-10 minutes.
6. Add Glycine to the sample at a proportion of 1:10 (100 µl to 1 ml of solution containing the fixed cells for example).
7. Mix by inversion of the tube four to five times. Incubate for 5 minutes at room temperature to stop the fixation. Work on ice from this point onwards.
8. Centrifuge at 600 x g for 10 minutes at 4°C (or higher speed depending on your cell type). We recommend the use of a swing-out rotor with soft settings for deceleration.
9. Aspirate the supernatant slowly and leave approximately 30 µl of the solution. Do not disturb the pellet. Take care to not remove these cross-linked cells.
10. Wash the cross-linked cells with 1 ml of ice cold HBSS containing protease inhibitor cocktail (PIC, 200x; final concentration 1x). Add 1 ml of HBSS and invert the tube four to five times to resuspend the cells.
11. When working with higher cell numbers (100 000 cells and more) you should **gently** vortex to completely resuspend the cells.
12. Centrifuge at 600 x g for 10 minutes at 4°C in a swing-out rotor with soft settings for deceleration (or higher speed depending on your cell type).
13. Discard all the supernatant (**no liquid should be left**) and keep the cell pellet on ice. The cell pellets can be stored at -80°C for up to 2 months.