diagenode
Innovating Epigenetic Solutions

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Cell Fixation Protocol for Histones

Cell amount:

Standard quantities per IP:

- ChIP on **histones**: >1 million 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)

Protocol:

The protocol below is intended for adherent cells. Collect suspension cells by centrifugation and continue with the protocol starting from step 6.

1. Pre-warm DPBS, culture medium and trypsin-EDTA at 37°C.
2. Remove the medium and rinse the cells with pre-warmed DPBS (10 ml for a 75 cm2 culture flask). Gently shake the flask for 2 min.
3. Remove the DPBS and add trypsin-EDTA to the culture flask to detach adherent cells from the bottom. Table 1 shows the required amount of trypsin for different flask surfaces. Gently shake the culture flask for 1-2 min or until the cells start to detach. The time needed may depend on the cell type. Do not continue trypsin treatment longer than necessary as prolonged treatment with trypsin may damage the cells. Regularly check if the cells start to detach.

Table 1		
Surface of flask	75 cm²	175 cm²
Trypsin-EDTA	1 ml	2 ml

4. Immediately add fresh culture medium to the cells when they are detached (Table 2). This will inactivate trypsin. Transfer cell suspension to a 50 ml tube.

Table 2		
Surface of flask	75 cm²	175 cm²
Culture medium	10 ml	20 ml

5. Rinse the flask by adding 10 ml of DPBS. Add this volume to your 50 ml tubes containing cells from point 4.
6. Centrifuge for 5 min at 1600 rpm and RT and remove the supernatant (or higher speed depending on your cell type). Make sure that the speed used results in cell pelleting without damaging the cell membrane.
7. Resuspend the cells in 20 ml of DPBS and count them. Collect the cells by centrifugation for 5 min at 1600 rpm and RT (or higher speed depending on your cell type).
8. Resuspend the cells in DPBS to obtain a concentration of up to 10 million cells per 500 µl of DPBS. If desired, the cell concentration can be decreased down to 1 million per 500 µl. Label 1.5 ml tubes and aliquot 500 µl of cell suspension in each tube.
9. Add 13.5 µl of formaldehyde 37% to each tube containing 500 µl of cell suspension. Mix by gentle vortexing and incubate for 8 min at room temperature to allow fixation to take place.
10. Add 57 µl of 1 .25 M Glycine to the cells to stop the fixation. Mix by gentle vortexing and incubate for 5 min at room temperature. Keep the cells on ice from this point onwards.
11. Collect the cells by centrifugation at 1600 rpm for 5 min and 4°C (or higher speed depending on your cell type). Discard the supernatant without disturbing the cell pellet.
12. Wash the cells twice with 1 ml of cold DPBS.
13. Freeze the cell pellets at -80°C.