diagenode
Innovating Epigenetic Solutions

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Cell Fixation Protocol for TF

Cell amount:

Standard quantities per IP:

- ChIP on **TFs**: >4 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 (PolII) and one negative (IgG) control are included in the workflow 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.

To allow an accurate estimation of the cell amount in the culture flasks for fixation, we recommend cultivating additional cells in parallel for exact cell counting. Please consider that different conditions (mutation, treatment,…) might influence the growth/proliferation behaviour.

Reagents :

- DPBS (Life technologies, 14190-094)
- 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)
- Fixation Buffer : 0,1 M NaCl, 1 mM EDTA, 0,5 mM EGTA, 50 mM HEPES pH 7,6

Protocol :

1. Dilute formaldehyde in Fixation Buffer to a final concentration of 11% (e.g. add 5 ml of a 37% formaldehyde solution to 11.8 ml Fixation buffer). For a T175 culture flask you will need ~2 ml of diluted formaldehyde.
2. Add 1/10 volume of the diluted formaldehyde directly to the cell culture medium.
3. Incubate the cells for 15 minutes at room temperature with gentle shaking.
4. Add 1/10 volume of Glycine to the cell culture medium to stop the fixation. Incubate for 5 minutes at room temperature with gentle shaking.

For adherent cells:

5. a. Remove the medium and wash the cells once with 20 ml of PBS. Keep everything at 4°C from now on.
6. a. Collect the cells by scraping in PBS.
7. a. Pellet the cells by centrifugation at 1,600 rpm for 5 minutes and 4°C and discard 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.
8. a. Freeze the cell pellet at -80°C.

For suspension cells:

5. b. Pellet the cells by centrifugation at 1,600 rpm and 4°C for 5 minutes (or higher speed depending on your cell type). Make sure that the speed used results in cell pelleting without damaging the cell membrane. Discard the cell culture medium.
6. b. Wash the cells once with PBS. Resuspend the cells in 20 ml of PBS, centrifuge at 1,600 rpm and 4°C for 5 minutes (or higher speed depending on your cell type) and discard the supernatant. Keep everything at 4°C from now on.
7. b. Freeze the cell pellet at -80°C.