January 2023

Cell Fixation Protocol with ChIP Cross Link Gold

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 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.

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
- PBS/$MgCl_2$: PBS with 1 mM $MgCl_2$
- ChIP Cross-Link Gold (Diagenode C01019027)

Protocol :

<u>For adherent cells :</u>

1. Remove the medium from the flask and wash the cells two times with PBS (20 ml for a 175 cm^2 culture flask).

2. Add 20 ml PBS/$MgCl_2$ to the cells (for a 175 cm^2 culture flask).
3. Add 80 µl of ChIP cross-link Gold to the flask. Immediately swirl to get the ChIP Cross-link Gold into solution. It may form a white precipitate after the addition of the ChIP Cross-link Gold. However it will be dissolved by gently mixing.
4. Incubate at RT for 30 min.
5. Remove the fixating solution and wash 2 times with PBS.
6. Add 20 ml of PBS to the cells.
7. 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).
8. Add 2 ml of diluted formaldehyde to the 20 ml of PBS. Incubate the cells for 15 minutes at room temperature with gentle shaking.
9. Add 1/10 volume of Glycine to the fixation solution to stop the fixation. Incubate for 5 minutes at room temperature with gentle shaking.
10. Remove the fixation solution and wash the cells once with 20 ml of PBS. Keep everything at 4°C from now on.
11. Collect the cells by scraping in PBS.
12. Pellet the cells by centrifugation at 1,600 rpm (or higher speed depending on your cell type) for 5 minutes and 4°C and discard the supernatant. Make sure that the speed used results in cell pelleting without damaging the cell membrane.
13. Freeze the cell pellet at -80°C.

<u>For suspension cells:</u>

1. Pellet the cells by centrifugation at 1,600 rpm (or higher speed depending on your cell type) and RT for 5 minutes. Discard the cell culture medium. Make sure that the speed used results in cell pelleting without damaging the cell membrane.
2. Wash the cells two times with PBS. Resuspend the cells in 20 ml of PBS, centrifuge at 1,600 rpm (or higher speed depending on your cell type) and RT for 5 minutes and discard the supernatant.
3. Add 20 ml of PBS/$MgCl_2$ to the cells and resuspend the cells.
4. Add 80 µl of ChIP cross-link Gold to the cells. Immediately mix to get the ChIP Cross-link Gold into solution. It may form a white precipitate after the addition of the ChIP Cross-link Gold. However it will be dissolved by gently mixing.
5. Incubate at RT for 30 min.
6. Pellet the cells by centrifugation at 3,000 rpm for 5 minutes and discard the fixating solution.
7. Wash 2 times with PBS. Resuspend the cells in 20 ml of PBS, centrifuge at 3,000 rpm and RT for 5 minutes and discard the supernatant.
8. Add 20 ml of PBS to the cells.
9. 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).
10. Add 2 ml of diluted formaldehyde to the 20 ml of PBS. Incubate the cells for 15 minutes at room temperature with gentle shaking.
11. Add 1/10 volume of Glycine to the fixation solution to stop the fixation. Incubate for 5 minutes at room temperature with gentle shaking.

12. Pellet the cells by centrifugation at 3,000 rpm and 4°C for 5 minutes. Discard the supernatant.
13. Wash the cells once with PBS. Resuspend the cells in 20 ml of PBS, centrifuge at 3,000 rpm and 4°C for 5 minutes and discard the supernatant. Keep everything at 4°C from now on.
14. Freeze the cell pellet at -80°C.