

Epigenetic Services ChIP Cell Fixation Protocol

- Fix cells for each cell population to be tested. To fix, add 1/10 volume of freshly-prepared Formaldehyde Solution* (see Reagents below) to the existing media in each container of cells (culture flask, plate or tube). Do NOT remove existing media. For example, to a flask containing 10 ml of media, add 1 ml of Formaldehyde Solution. Cap and agitate for exactly 15 minutes at room temperature.
- Stop the fixation by adding 1/20 volume Glycine Solution* to the existing media in each container. For example, if the flask from Step 1 now contains 11 ml, add 0.55 ml 2.5 M glycine. Let sit at room temperature for 5 minutes. After the glycine incubation, if the cells are adherent, scrape them thoroughly from the culture surface.
- Wash cells by transferring contents of each container to a conical tube (15 ml or 50 ml tube, depending on the volume). Keep samples on ice for the remainder of the procedure. Centrifuge tubes at 800 x g in a refrigerated centrifuge for 10 minutes to pellet the cells. Remove the supernatant and re-suspend cells in 10 ml chilled PBS-Igcpal* per tube by pipetting up and down. If cells from any one population are contained in multiple centrifuge tubes, combine them at this step.
- Centrifuge tubes again as before to pellet the cells. Remove the supernatant, then add 10 ml chilled PBS-Igcpal* to each tube. Add 100 µl PMSF (100 mM in ethanol*; final concentration will be 1 mM) to each tube and pipet up and down to resuspend the cells.
- Centrifuge tubes a third time to pellet the cells, and carefully remove supernatant completely from cell pellets.
- Snap-freeze cell pellets on dry ice and store at -80°C.
- Ship cells on dry ice to Active Motif. Follow the instructions shown on our Sample Submission Form, which can be downloaded at: www.activemotif.com/sample-submission.

| Reagents* | Final concentration | Per 20 ml |
|--|----------------------------|-------------------|
| 1. Formaldehyde Solution (to be prepared fresh before use): | | |
| 37% Formaldehyde (e.g. Sigma #F-8775) | 11% | 6 ml |
| 5 M NaCl | 0.1 M | 0.4 ml |
| 0.5 M EDTA, pH 8.0 | 1 mM | 40 µl |
| 1 M HEPES, pH 7.9 | 50 mM | 1 ml |
| H ₂ O | | to 20 ml |
| (Note: NaCl, EDTA, and HEPES should be molecular biology grade.) | | |
| 2. Glycine Solution | | |
| | | Per 20 ml |
| Glycine, MW 75 (e.g. Sigma #G-7403) | 2.5 M | 3.75 g |
| H ₂ O | | to 20 ml |
| 3. PBS-Igcpal | | |
| | | Per 100 ml |
| PBS, pH 7.4 (e.g. ThermoFisher #10010023) | ~1X | 100 ml |
| 100% Igcpal CA-630 (e.g. Sigma #I-8896) | 0.5% | 0.5 ml |
| 4. PMSF (e.g. Sigma #P-7626) | | |
| Prepare at 100 mM in ethanol and store at -20°C. | | |
| (Note: PMSF Phenylmethanesulfonyl fluoride.) | | |