Services ChIP-Seq Standard Formaldehyde Preparation for Cells

Active Motif Services requires a minimum of 4-5 million cells per IP. However, more cells are encouraged and ≥10 million can be beneficial for transcription factors. Fix ALL cells in a population at once, scaling up as required. If the volume required is more than the capacity of the fixation vessel/tube, please split cells into multiple vessels/tubes and combine them into a single tube after adding chilled PBS-IGEPAL as noted after Step 3.

Required Reagents and Materials:

- PBS phosphate-buffered saline without Ca^{2+}/Mg^{2+}, e.g. ThermoFisher cat #10010023
- 37% formaldehyde, e.g. Sigma #F-8775
- Glycine, e.g. Millipore Sigma #G-7403, MW 75.07
- 5 M NaCl, e.g. Millipore Sigma # S6546-1L
- 0.5 M EDTA, pH 8 (e.g. Millipore Sigma cat# 20-158)
- 1 M HEPES, pH 8
- 100% Igepal CA-630 (e.g. Sigma #I-8896)
- PMSF Phenylmethanesulfonyl fluoride (e.g. Sigma #P-7626)
- H2O
- Ethanol
- 1.5 ml, 15 ml, and 50 mL centrifuge tubes
- Bucket of ice
- Dry ice

Prepare Buffers

Prepare buffers below according to the number of cells being fixed in a population. Scale up as required. The tables have been provided for convenience.

11% Formaldehyde Solution

Prepare **fresh** 11% Formaldehyde Solution by adding the following to a 50 mL conical tube.

11% Formaldehyde Solution	*Final Concentration*	*10 million cells*	*20 million cells*
37% Formaldehyde	11%	3 ml	6 ml
5 M NaCl	0.1 M	200 µL	400 µL
0.5 M EDTA, pH 8	1 mM	20 µL	40 µL
1 M HEPES, pH 8	50 mM	500 µL	1 ml
H_2O		6.28 ml	12.56 ml
Total		10 ml	20 ml

2.5 M Glycine Solution

Prepare 2.5 M Glycine Solution by adding the indicated amount of glycine to a 15 ml conical tube and bringing up the volume. Place at room temperature.

2.5 M Glycine Solution	*10 million cells*	*20 million cells*
Glycine	1.03 g	2.06 g
H_2O	to 5.5 ml	to 11 ml

0.5% PBS-Igepal Solution

Prepare 0.5% PBS-Igepal Solution by adding the following to a 50 mL conical tube. Place at 4°C.

0.5% PBS-Igepal	*Amount*	
PBS	100 ml	
100% Igepal	0.5 ml	
100% Igepal	0.5 ml	0.1 ml
PBS	100 ml	20 ml

100 mM PMSF Solution

Prepare 100 mM PMSF Solution by adding the following to a 50 mL conical tube. Place at 4°C.

100 mM PMSF	*Amount*
PMSF	17.42 mg
Ethanol	To 1 ml

Protocol:

1. To fix the cells, add 1/10 volume of freshly prepared 11% Formaldehyde Solution 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.

2. 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 it sit at room temperature for 5 minutes. After the glycine incubation, if the cells are adherent, scrape them thoroughly from the culture surface.

The following steps should be done on ice.

3. Wash cells by doing the following:
   I. Transfer the contents of each container to a conical tube (15 ml or 50 ml tube, depending on the volume).
   II. Centrifuge tubes at 800 x g in a refrigerated centrifuge for 10 minutes to pellet the cells.

III. Remove the supernatant.
IV. Per tube, resuspend cells in 10 ml chilled PBS-Igepal by pipetting up and down.

If the cells from any one population are contained in multiple tubes, combine them into one tube at this point.

4. Centrifuge tube at 800 x g in a refrigerated centrifuge for 10 minutes to pellet the cells.
5. Remove the supernatant.
6. Add 10 ml chilled 0.5% PBS-Igepal to each tube.
7. Add 100 µL of 1 mM PMSF (100 mM in ethanol*; final concentration will be 1 mM) to each tube and pipet up and down to resuspend the cells.
8. Centrifuge tubes at 800 x g in a refrigerated centrifuge for 10 minutes to pellet the cells.
9. Carefully remove supernatant completely from cell pellet.
10. Snap-freeze cell pellets on dry ice and store at -80°C.

Best Practices for sending samples to Active Motif

- Seal top of tube with parafilm to avoid tube from opening during transit
- Ensure that there is enough dry ice in package for transport
- Avoid shipping over a weekend or for Saturday delivery
- Ship samples Monday through Wednesday
- Ensure that a complete sample submission form is included in the shipment