DISCOVER-Seq Cell Fixation Protocol

We require 4-5 million cells per IP. Fix ALL cells at once, such that there is 1 mL of volume for every 1 million cells. If 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 in Step 3.

1. 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.
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 sit at room temperature for 5 minutes. After the glycine incubation, if the cells are adherent, scrape them thoroughly from the culture surface.
3. 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-Igepal* per tube by pipetting up and down. If cells from any one population are contained in multiple centrifuge tubes, combine them at this step.
4. Centrifuge tubes again as before to pellet the cells. Remove the supernatant, then add 10 ml chilled PBS-Igepal* 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.
5. Centrifuge tubes a third time to pellet the cells, and carefully remove supernatant completely from cell pellets.
6. Snap-freeze cell pellets on dry ice and store at -80°C.

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_2O		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_2O		to 20 ml

3. PBS-Igepal		**Per 100 ml**
PBS, pH 7.4 (*e.g.* ThermoFisher #10010023)	~1X	100 ml
100% Igepal 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.)

DISCOVER-Seq Tissue Preparation

If you are planning on submitting tissues for ChIP-Seq services, freeze tissue according to one of the protocols below. Tissue requirements for ChIP-Seq services are 100- 500mg*.

* For fatty tissue such as mammary gland, Active Motif recommends 1g per sample.

Consumables

- Cryogenic vial(s) ***or*** 2 ml low-bind microcentrifuge tube
- Liquid Nitrogen
- Dry ice

Protocol A: Liquid Nitrogen

1. Excise the tissue from the animal and place in a cryogenic vial or microcentrifuge tube
2. Immediately submerge tube in liquid nitrogen for 2 minutes
3. Store at -80ºC

Protocol B: Dry Ice

1. Excise the tissue from the animal and place in cryogenic vial or microcentrifuge tube
2. Immediately place tube on dry ice for 15 minutes
3. Store at -80ºC

Best Practices for sending samples to Active Motif

- Avoid overfilling tubes with tissue as this makes it very difficult to extract samples from tube
- 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 completed sample submission form is included in the shipment