

## Cell Fixation Protocol

### Procedure

- 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 11% 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 set 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-Igepal\* per tube by pipetting up and down. If cells from any one population are contained in multiple centrifuge tubes, combine together at this step.
- 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.
- Centrifuge tubes a third time to pellet the cells, and carefully 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 at 1914 Palomar Oaks Way, Ste 150, Carlsbad, CA 92008.

Reagents*	Final concentration	Per 20 ml
1. Formaldehyde Solution (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	1 mM	40 µl
1 M HEPES, pH 7.9	50 mM	1 ml
H <sub>2</sub> O		to 20 ml
(Note: NaCl, EDTA, and HEPES should be molecular biology grade)		
2. Glycine Solution		
Glycine, MW 75 (e.g. Sigma #G-7403)	2.5 M	3.75 g
H <sub>2</sub> O		to 20 ml
		<b>Per 100 ml</b>
3. PBS-Igepal		
Phosphate-buffered saline (e.g. Invitrogen #10010-023)	~1X	100 ml
100% Igepal CA-630 (e.g. Sigma #I-8896)	0.5%	0.5 ml
(Note: Igepal CA-630 is chemically indistinguishable from Nonidet P-40 or NP-40)		
4. PMSF (e.g. Sigma #P-7626)		
Prepared at 100 mM in ethanol and stored at -20 C		
(Note: PMSF is Phenylmethanesulfonyl fluoride)		