

## Cell Fixation Protocol

### Procedure

1. 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.
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 set 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 together 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 supernatant completely from cell pellets.
6. Snap-freeze cell pellets on dry ice and store at -80 C.
7. 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)		