TIP-ChIP
Cell Sample Fixation Protocol

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For best results Active Motif recommends fixing 300,000 to 1,000,000 cells per well. While less than 300,000 cells will be accepted, there is an increased likelihood of the duplication rate increasing to a point where analysis is not possible. The protocol below is designed for fixing cells in a 96 well round bottom culture plate. A Corning® 96-well Clear Round Bottom TC-treated Microplate (Product No. 3799) is recommended. Cells can either be grown in the plate, or aliquoted into the plate directly before fixation.

	Minimum	Recommended
Histone Modifications	300K	300K-1M
Transcription Factors	1M	1M

Required Reagents and Equipment

- Liquid nitrogen
- 10X Cell Fixation Buffer, requires:
  - 16% Formaldehyde Solution (e.*g*. Pierce™ Catalog No. 28906)
  - 5 M NaCl (e.*g*. Millipore Sigma Catalog No. S6546-1L)
  - 250 mM EDTA, pH 8.0 (e.*g*. Millipore Sigma Catalog No. 20-158)
  - 1 M HEPES, pH 7.5 (e.*g*. Thermo Fisher Scientific Catalog No. J60717.AP)
- 2.5M Glycine solution
- 1X PBS
- Swing bucket centrifuge
- Corning® 96-well Clear Round Bottom TC-treated Microplate
- Multichannel pipette
- Aluminum sealing foil

See below for 10X Cell Fixation Buffer recipe

Protocol

1. Prepare the 10X Cell Fixation Buffer according to the instructions below. The formaldehyde should be added to the buffer directly before use, do not prepare more than 4 hours in advance.
2. Count cells and check viability. Viability should be at least 80%.
3. Adjust cell concentration with warm or room temperature media so that 100 µL contains the cell number desired per well. For example, 3.0 x 10^6 cells/mL for 300,000 cells per well or 10 x 10^6 cells/mL for 1 million cells per well. Ensure that the total volume of cells in each well is consistent across the plate and does not exceed 110 µL.
4. Aliquot 100 µL of cells into each well of a 96 well plate.
5. Add 10 µL (1/10 volume) of the 10X Cell Fixation Buffer to each well.

Note: The amount of time the cells are exposed to the fixation buffer is critical, please use a multichannel pipette to carry out this step, and step 7, as quickly as possible.

6. Place plate on a nutator at room temperature for 10 minutes.
7. Add 5.5 µL (1/20 volume) 2.5M glycine to each well to stop fixation, pipetting up and down to mix.

Note: All remaining steps must be performed on ice or in a cold room.

8. Incubate plate on ice for at least 5 minutes.
9. Spin plate in a swing bucket centrifuge at 800 x *g* for 5 minutes at 4°C.
10. Remove supernatant by flicking. For best results, quickly and without hesitation invert plate and flick supernatant off in one motion.
11. Add 100 µL of cold 1X PBS to each well. Do not disturb the cell pellet. Incubate on ice for 2 minutes.
12. Spin plate in a swing bucket centrifuge at 800 x *g* for 5 minutes at 4°C.
13. Remove supernatant by flicking. For best results, quickly and without hesitation invert plate and flick supernatant off in one motion.
14. Add 100 µL of cold 1X PBS to each well.
15. Spin plate in a swing bucket centrifuge at 800 x *g* for 5 minutes at 4°C.

Note: Collect liquid nitrogen while plate is spinning if not already collected.

16. Remove supernatant by flicking. For best results, quickly and without hesitation invert plate and flick supernatant off in one motion.
17. Seal plate with aluminum sealing foil, we recommend TempPlate® Sealing Foil from USA Scientific.
18. Fill a Styrofoam ice bucket (or any liquid nitrogen safe container) with liquid nitrogen so that the bottom is covered with at least 1 inch of liquid nitrogen.
19. Carefully place the plate in liquid nitrogen so that it floats on top for at least 20 seconds. The plate does not need to be completely submerged.
20. Transfer to storage at -80°C until ready to ship.

10X Cell Fixation Buffer

Below is a recipe for making 1.35 mL of 10X Cell Fixation Buffer, using a convenient 1 mL ampule of Pierce™ 16% Formaldehyde (w/v), Methanol free, Catalog No. 28906. All reagents should be molecular biology grade.

Reagent	Volume	Final Concentration
16% Formaldehyde Solution	928 µL	11%
5 M NaCl	27 µL	100 mM
250 mM EDTA, pH 8.0	5.4 µL	1 mM
1 M HEPES, pH 7.5	67.5 µL	50 mM
Water	322.1 µL	
Total Volume	**1350 µL**	