ChIP-IT® Express: Supplemental Protocol for Suspension Cells

Cell Fixation for Suspension Cells

1. Cells for a typical expermiment: 1 X 10^7 cells grown in suspension. Add formaldehyde diluted directly into tissue culture media to a final concentration of 1% (1.35 mL of 37% formaldehyde into 50 mL media). Incubate suspension cells on a stir plate or gently invert the tube 2-3 times during cross-linking for 5-10 minutes at room temperature.
   NOTE: Cross-linking for longer periods of time tends to cause cells to form into a giant cross-linked aggregare that cannot be efficiently sheared.
2. Pellet cells by centrifugation for 10 minutes at 2,500 rpm at 4°C. Discard the supernatant carefully.
3. Wash cells by gently resuspending the pellet with 5 mL ice-cold PBS.
4. Pellet cells by centrifugation for 10 minutes at 2,500 rpm at 4°C. Do NOT discard the supernatant.
5. Stop the cross-linking reaction by adding 5 mL of 2X Glycine Stop-Fix Solution. Gently invert the tube 2-3 times for 5-10 minutes at room temperature.
6. Pellet cells by centrifugation for 10 minutes at 2,500 rpm at 4°C. Discard the supernatant carefully.
7. Wash cells by gently resuspending the pellet with 5 mL ice-cold PBS.
8. Pellet cells by centrifugation for 10 minutes at 2,500 rpm at 4°C. Discard the supernatant carefully.
9. At this point, the protocol can be continued or the cell pellet can be frozen at -80°C.
10. Thaw pellet (if necessary) and resuspend cells in 1 mL ice-cold Lysis Buffer (suuplemented with 5 µL Protease Inhibitor Cocktail + 5 µL PMSF). Incubate on ice for 30 minutes.
11. Transfer cells to an ice-cold Dounce homogenizer. Perform 10 passes with the Dounce homogenizer.
    a. To ensure cell lysis, sample 10 µL of the cell lysate and look at it under a phase contrast microscope using a hemocytometer. Released nuclei visually look like little dots (periods) in a field of tiny irregular debris. Poorly lysed samples contain intact cells that have a dark central region (nucleus) surrounded by a halo of the less dense cytoplasm. Look at cells before the lysis step and after Douncing for easier identification of the nuclei versus whole cells — it is sometimes difficult to determine. If the cells are not lysed, then do an additional 10 passes with the Dounce homogenizer until the cells are lysed.
    b. Suggested homogenizer: We recommend a small pestle clearance of 0.013 - 0.064 mm, such as Active Motif's Cat. No. 40401 for 1 mL capacity, or Cat. No. 40415 for 15 mL capacity. It is designed for cellular work, in which the nucleus remains intact after homogenization with the tightest pestle.

12. Transfer lysates to a 1.7 mL microcentrifuge tube and centrifuge at 5,000 rpm (approximately RCF 2,400) for 10 minutes at 4°C to pellet the nuclei. Discard supernatant and resuspend pellet in the appropriate volume of supplemented Shearing Buffer (Sonication) or Digestion Buffer (Enzymatic).
13. At this point, you can continue on with the Active Motif ChIP-IT® Express Manual: Section B1: Shearing by Sonication step 3 or Section B2: Enzymatic Shearing step 3.

Technical Support

If you need assistance at any time, please contact Active Motif Technical Support at tech_service@activemotif.com.