Quick Guide: Shearing Chromatin with PIXUL™

This Quick Guide provides recommendations for researchers fragmenting chromatin with the PIXUL Multi-Sample Sonicator using the PIXUL Chromatin Shearing Kit (cat. no. 53132). PIXUL sonication is compatible with a wide range of fixed cells (10,000 – 5,000,000) with no changes in sonication efficiency or processing time.

IMPORTANT: *PIXUL sonication requires using PIXUL 96-well round bottom plates (cat. no. 53139). Using any other plates may result in inefficient sonication, may damage the instrument transducers, and will void the instrument warranty.*

Buffer Preparation Before Starting

1X PBS: 4.5 mL 10X PBS + 40.5 mL nuclease-free water
Fixation Solution stock: 293 µL nuclease-free water + 90 µL Fixation Buffer + 867 µL 16% methanol-free formaldehyde
Fixation Solution in PBS (working solution): 1 mL Fixation Solution stock + 10 mL 1X PBS
1X Stop Solution in PBS: 0.6 mL Stop Solution + 11.4 mL 1X PBS
Cell Shearing Buffer with Inhibitors: 10 mL Cell Shearing Buffer + 100 µL Protease Inhibitor Cocktail

Cell Fixation

1. Culture cells directly in PIXUL 96-well round bottom plate (cat. no. 53139) or cell culture dish or flask of your choice.
2. If using the PIXUL 96-well plates for cell culture, carefully aspirate media and add 100 µL Fixation Solution. If not using the PIXUL 96-well plates for cell culture, collect cells and resuspend the appropriate number of cells in 100 µL Fixation Solution and transfer to the PIXUL 96-well plate.
3. Incubate for 10 minutes at room temperature. If you are working with suspension cells, collect cells by centrifugation.
4. Aspirate Fixation Solution and add 100 µL Stop Solution.
5. Incubate for 5 minutes at room temperature. If you are working with suspension cells, collect cells by centrifugation.
6. Aspirate Stop Solution and wash the cells twice with 100 µL 1X PBS. If you are working with suspension cells, collect cells by centrifugation before aspiration.

PIXUL Sonication of Fixed Cells

1. Aspirate PBS and add 100 µL Cell Shearing Buffer with Inhibitors to the fixed cells and seal the plate with a PIXUL plate seal.
2. Turn on and run the instrument as specified in the PIXUL Multi-Sample Sonicator User Manual.
3. We recommend using the following parameter specifications as a starting point, and adjusting Process Time to optimize for your specific sample and application:

Sonication Parameter	Setting
Pulse [N]	50
PRF [kHz]	1.00
Process Time [min]	36:00
Burst Rate [Hz]	20.00

Analysis of Sonicated Chromatin

Remove 10 µL of the sonicated chromatin and use the PIXUL Chromatin Input Preparation Kit (cat. no. 53134) to determine yield and sonication efficiency. We recommend shear-ing chromatin to 200-600 bp in length for ChIP assays.

Using Chromatin Sheared Using PIXUL in Chromatin Immunoprecipitation Assays

Chromatin sheared with PIXUL is compatible with many ChIP protocols, but for best results we recommend performing ChIP assays using the ChIP-IT High Sensitivity® kit (cat. no. 53040).

Technical Support

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