Quick Guide: Shearing Chromatin from Frozen Tissues with PIXUL™

This Quick Guide provides general recommendations for researchers fragmenting chromatin with the PIXUL™ Multi-Sample Sonicator (Cat. No. 53130). Please contact Active Motif for specific details and examples.

IMPORTANT: *PIXUL sonication requires using PIXUL 96-well round bottom plates (cat. no. 53139).*

1. Use razor blade to slice tissue or apparatus to core tissue into 1 mm^3 or 1 mg pieces which approximately amounts to 0.5-1 million cells.
2. Place individual pieces in individual wells of microplate, add 100 µL 1% formaldehyde in PBS, shake 15 seconds, incubate 20 minutes (mins) at room temperature (RT).
3. Remove supernatant, add 200 µL PBS/glycine (125 µM), incubate 5 mins, RT. Remove supernatant, wash with 200 µL PBS.
4. Add 100 µL Chromatin Shearing Buffer (in kit Cat. No. 53132) per well, seal plate and place plate inside PIXUL.
5. Run Sonication Program: Pulse 50, PRF 1kHz, Burst Rate 20 Hz, Time = 6 min X 4 cycles. Times may vary depending on sample types and applications.

Alternative protocol:

Recommendations for researchers fragmenting chromatin with the PIXUL Multi-Sample Sonicator using the PIXUL Chromatin Shearing Kit (cat. no. 53132). This protocol has been used with human and mouse tissues where each tissue sample represents 10-50 mg of tissue, or a 1 mm3 cube, which was homogenized in 1 mL Cell Shearing Buffer and split over 8 wells in 1 column of the PIXUL 96-well plate.

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Prepare before starting:

- Using a clean razor blade or scissors, cut the end of a 1 mL pipette tip (for each sample)
- 15 mL conical tubes and 60 mm petri dishes (1 per sample).
- 1X PBS pH 7.4 (cold): 4.5 mL 10X PBS + 40.5 mL nuclease-free water
- Fixation Solution (prepare in hood): # samples X 10 mL 1X PBS pH 7.4 (cold) + # of samples X 280 µL 37% formaldehyde
- Wash Solution: 50 mL 1X PBS + 250 µL IGEPAL-CA630 (or NP-40) + 500 µL 100 mM PMSF
- 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 + 100 µL 100 mM PMSF
- Homogenization tool (Omni Beadruptor was used here. If a different homogenizer is used, follow the manufacturer's instructions)

Fixation

1. Fix up to 3 samples at a time, keep other samples on dry ice before fixing. Per sample, transfer 10 mL Fixation Solution to 60 mm petri dish on ice.
2. Transfer tissue sample to petri dish containing Fixation Solution, immerse sample and cut it into 1 mm^3 cubes with a razor blade on ice. If the sample is frozen in its storage tube, use the pre-cut 1 mL pipette tip and pipette some Fixation Solution into the tube. Use a 200 µL tip to loosen and dislodge the sample, and transfer it to the dish.
3. Transfer the sample from the dish to a 15 mL conical tube using the pre-cut pipette tip. Incubate with rotation at room temperature for 15 minutes.
4. Add 515 µL Stop Solution and incubate at room temperature for 5 minutes while rotating.

Homogenization

5. Decant Fixation Solution from sample.
6. Transfer tissue sample to petri dish containing 10 mL Wash Solution.
7. To a tube for homogenization, add 1 mL Cell Shearing Buffers with Inhibitors, and homogenize. We use 6 X 2.4 mm or 3 X 5 mm stainless steel beads on the Omni Beadruptor, 4 m/s for 20 seconds. Tough tissue such as uterus, ovary, colon, or adipose samples may need an additional cycle.
8. Count cells, and proceed to sonication. Keep a 50 µL aliquot for an unsonicated control, store at -80°C.

PIXUL Sonication of Tissue Samples

1. Aliquot 100 µL sample per well 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

4. After sonication, briefly spin plate to collect sonicated samples, and pool wells together as needed for each sample. Centrifuge samples at 4°C for 3 minutes at maximum speed to pellet any insoluble material. Move the supernatant to a clean tube.

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 shearing chromatin to 200-600 bp in length for ChIP assays

Using Chromatin Sheared with 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) or High Throughput ChIP-IT® Kit (cat. no. 53146).

Technical Support

If you need assistance at any time, please call or send an e-mail to Active Motif Technical Service at one of the locations listed below.

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