PIXUL™ Chromatin Shearing Kit Manual

Catalog No. 53132

(version A1)

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The manufacturer of this documentation is Active Motif, Inc.

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Overview

Highly consistent shearing of chromatin is critical for successful downstream chromatin immunoprecipitation assays. Manual shearing with a probe sonicator or shearing with an inconsistent high-throughput sonicator can lead to sample-to-sample variability.

The PIXUL Chromatin Shearing Kit delivers highly consistent average fragment lengths across entire plates time after time. This kit is compatible with a wide range of fixed cells (10,000 - 5,000,000) with very low standard deviations in sonication efficiency.

PIXUL Advantages

- Process 1 - 96 samples in parallel
- Extremely consistent sonication
- Up to 12 different sonication conditions per run

product	format	catalog no.
PIXUL™ Chromatin Shearing Kit	1 X 96 reactions	53132

Kit Components and Storage

The kit contains sufficient reagents to sonicate 96 wells in one run. The reagents in this kit have multiple storage temperatures. Please store components according to the storage conditions below. All reagents are guaranteed stable for 6 months from date of receipt when stored properly.

Reagents	Quantity	Storage
Fixation Buffer	75 µL	4°C
Stop Solution	560 µL	RT
10X PBS	6 mL	4°C
Protease Inhibitor Cocktail	2 X 100 µL	-20°C
Cell Shearing Buffer	11 mL	RT
PIXUL™ 96-well plate with sealer	1 unit	RT

Additional Materials Required

- 100% Ethanol
- Nuclease-free water
- 16% methanol-free formaldehyde
- 1X PBS
- PIXUL Multi-Sample Sonicator (Cat. No. 53130)
- Microcentrifuge tubes
- PCR tube strips (300 µL well volume capacity)
- Centrifuge to spin 96-well plate
- Thermal cycler or other 37°C incubator
- 96-well plate magnet or bar magnet
- Single channel and multi-channel pipettors and tips
- Hemocytometer and light microscope

IMPORTANT: PIXUL sonication requires the use of PIXUL 96-well round bottom plates. These plates are available as Active Motif Catalog No. 53139, and these are the same plate as Corning Catalog No. 3799. Using any other plates will result in inefficient sonication, may damage the instrument, and will void the instrument warranty.

Buffer Preparation

Prepare the following buffers before starting the protocol.

1X PBS
- 4.5 mL 10 X PBS
- 40.5 mL nuclease-free water

Fixation Solution (Stock Solution)
- 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 Solution)
- 10 mL 1X PBS

1X Stop Solution
- 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 the PIXUL 96-well round-bottom plate or cell culture dish or flask of your choice.
2. If you are using the PIXUL 96-well plates for cell culture, carefully aspirate media and add 100 µL Fixation Solution (Working Solution).
3. If you are not using the PIXUL 96-well plate for cell culture, collect cells, and resuspend the appropriate number of cells in 100 µL Fixation Solution (Working Solution) and transfer to the PIXUL 96-well plate.
4. Incubate for 10 minutes at room temperature. If you are working with suspension cells, collect cells by centrifugation.
5. Aspirate Fixation Solution and add 100 µL 1X Stop Solution.
5. Incubate for 5 minutes at room temperature. If you are working with suspension cells, collect cells by centrifugation.
6 Aspirate 1X 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.

   Note: All wells lacking sample in the columns being sonicated MUST be filled with liquid (water, coupling buffer, etc.) prior to starting the run.

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:

   Labile marks, like phosphorylated epitopes, may be preserved better by discontinuous sonication (for example, rather than 30 minutes, 4 rounds of 4 - 6 minutes each, where the PIXUL Coupling Fluid is allowed to circulate and cool between runs).

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

Note: The instrument software will not allow settings that could damage the transducers. The upper limits are a maximum Pulse of 50 or a maximum PRF of 1.00. We recommend a minimum Burst Rate of 20.00. Varying the Process Time is typically the only parameter that needs adjusting, with longer Time needed for difficult samples such as adipose tissue.

Analysis of Sonicated Chromatin

Remove 10 µL of the sonicated chromatin and use the PIXUL Chromatin Input Kit (Cat. No. 53134) to determine yield and sonication efficiency. We recommend shearing chromatin to 200 - 500 bp in length for ChIP assays.

Using Chromatin Sheared Using PIXUL in ChIP 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 Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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