

Quick Guide: PIXUL™ Chromatin Input Prep Kit

This Quick Guide provides instructions for analyzing chromatin yield and sonication efficiency following chromatin shearing with a PIXUL Multi-Sample Sonicator using the PIXUL Chromatin Input Preparation Kit (cat. no. 53134).

Buffer Preparation Before Starting

TE-RNase: 95 μ L Low EDTA TE + 5 μ L RNase A (10 μ g/ μ L)

EB-PK: 95 μ L Elution Buffer AM4 + 5 μ L Proteinase K (10 μ g/ μ L)

80% Ethanol: 4.8 mL 100% ethanol + 1.2 mL nuclease-free water

Reverse Crosslinking

1. Remove 10 μ L of the chromatin that has been sonicated with the PIXUL Chromatin Shearing Kit (cat. no. 53132) and transfer to a PCR tube, strip-well, or 96-well plate.
2. Add 10 μ L TE-RNase to each PCR tube and incubate for 30 minutes at 37 °C.
3. Add 10 μ L EB-PK to each PCR tube and incubate for 30 minutes at 55 °C, followed by 90 minutes at 65 °C.

DNA Purification Using SPRI Beads

1. Allow sample to cool to room temperature and add 36 μ L (1.2 volumes) of SPRI beads to each tube.
2. Pipette the bead-lysate mixture 10 times to mix and incubate for 5 minutes at room temperature.
3. Apply magnet to tube or plate to collect beads for 5 minutes, and then aspirate supernatant.
4. Keeping the tube or plate on the magnet, add 180 μ L of 80% ethanol and incubate for 30 seconds at room temperature.
5. Aspirate supernatant and perform a second wash with 180 μ L of 80% ethanol.
6. Completely aspirate the supernatant and air dry the beads until they are no longer shiny (2-5 minutes).
7. Remove tube or plate from the magnet and elute DNA by adding 10 μ L of Low EDTA TE.
8. Pipette the beads 10 times and incubate for 3 minutes at room temperature.
9. Place tube or plate back on the magnet for 5 minutes to collect beads.
10. Carefully remove supernatant containing the eluted DNA and transfer to a new tube or plate.

Analyze Sonicated DNA

Analyze chromatin yield using a spectrophotometric (Nanodrop) or fluorometric (Qubit) method of your choice and analyze fragmentation efficiency using either agarose gel electrophoresis, Agilent TapeStation, or Agilent Fragment Analyzer. We recommend shearing chromatin to 200-600 bp in length for ChIP assays.

Technical Support

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