PIXUL™ Chromatin Input Prep Kit Manual

Catalog No. 53134

(version A1)

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The manufacturer of this documentation is Active Motif, Inc.

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Overview

This kit is designed to analyze yield and sonication efficiency of the chromatin sheared with the PIXUL™ Chromatin Shearing Kit (Cat. No. 53132) using the PIXUL™ Multi-Sample Sonicator (Cat. No. 53130).

The kit contains sufficient reagents to prepare ChIP-ready chromatin for 96 IP reactions.

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 Input Prep Kit	1 X 96 reactions	53134

Kit Components and Storage

The kit contains sufficient reagents to prepare analyze ChIP-ready chromatin for 96 IP reactions. 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
SPRI Beads	3.98 mL	4°C
Elution Buffer AM4	1.1 mL	RT
Proteinase K	56 µL	-20°C
RNase A	2 X 45 µL	-20°C
Low EDTA TE	2 X 1.2 mL	RT

Additional Materials Required

- 100% Ethanol
- Nuclease-free water
- 1X PBS
- Microcentrifuge tubes
- PCR tube strips (300 µL well volume capacity)
- Centrifuge to spin 96-well plate
- Thermal cycler
- 96-well plate magnet or bar magnet
- Single channel and multi-channel pipettors and tips
- Hemocytometer and light microscope

PIXUL Chromatin Input Prep Kit Protocol

Buffer Preparation

1. Prepare TE-RNase by combining 95 µL Low EDTA TE and 5 µL RNase A (10µg/µL).
2. Prepare Elution Buffer with Proteinase K (EB-PK) by combining 95 µL Elution Buffer AM4 with 5 µL Proteinase K (10µg/µL).
3. Prepare 80% ethanol (4.8 mL 100% ethanol + 1.2 mL nuclease-free water).

Reverse Crosslinking

1. Remove 10 µL 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 µL TE-RNase to each sample and incubate for 30 minutes at 37°C.
3. Add 10 µL EB-PK to each sample and incubate for 30 minutes at 55°C, followed by 90 minutes at 65°C.

DNA Purification using SPRI Beads

Take SPRI Beads out of 4°C and warm to room temperature by simply placing the vial of SPRI beads on the benchtop.

1. Add 0.5 µL RNase A to each well and incubate for 30 minutes at 37°C.
2. Add 5 µL Proteinase K to each well and incubate for 30 minutes at 55°C.
3. Add 120 µL (1.2 volumes) of SPRI Beads to each well. Pipette the bead-lysate mixture 10 times to mix, and incubate for 5 minute at room temperature.
4. Place plate on a magnet for 5 minutes to collect beads, and then aspirate supernatant.
5. Keeping the plate on the magnet, add 180 µL of 80% ethanol and incubate for 30 seconds at room temperature.
6. Aspirate the supernatant and perform a second wash with 180 µL of 80% ethanol.
7. Completely aspirate the supernatant and air dry the beads until they are no longer shiny (2-5 minutes).
8. Remove the plate from the magnet and elute DNA by adding 50 µL of Low EDTA TE.
9. Pipette the beads 10 times and incubate for 3 minutes at room temperature.
10. Place tube or plate back on the magnet for 5 minutes to collect beads.
11. Carefully remove eluted DNA in the supernatant and transfer to a new tube or plate.
12. Analyze gDNA 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. For most applications, we recommend shearing DNA to 200 - 600 bp.

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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