

EPIGENOMICS SERVICES

Sample Preparation Guide for HiChIP



SCOPE Crosslinking Principle · Suspension Cell Crosslinking · Adherent Cell Crosslinking · Tissue Crosslinking · Recommended Number of Cells

These are general sample preparation recommendations. Final requirements are subject to the project confirmation.

01 Crosslinking Principle

Formaldehyde crosslinking fixes interactions between proteins and genomic DNA. These interactions can be strong or weak, and transient or long-lasting. Depending on the experiment, strong interactions may require a shorter crosslinking time or no crosslinking. Longer crosslinking times can make subsequent chromatin fragmentation by sonication more difficult. Crosslinking time can therefore be adjusted for different experiments, usually within 5–30 minutes; most experiments use 10 minutes.

02 Suspension Cell Crosslinking

1. Resuspend up to 10 million cells in 10 mL of 1× PBS containing 270 µL of 37% formaldehyde (final formaldehyde concentration: 1%). Crosslink for 10 minutes at room temperature using a vertical mixer. Centrifuge at 500 × g for 5 minutes and wash twice with 2 mL of 1× PBS.
2. Add 530 µL of 2.5 M glycine. Prepare the glycine solution using 9.38 g glycine and 50 mL ddH₂O, dissolving at 37 °C. Neutralize for 5 minutes at room temperature using a vertical mixer, then place on ice for 2 minutes.
3. Centrifuge at 500 × g for 5 minutes at 4 °C. Discard the PBS supernatant.
4. Resuspend the cells in 1 mL of 1× PBS using a pipette, transfer to a 1.5 mL centrifuge tube, and centrifuge at 500 × g for 5 minutes. Remove the supernatant and retain the cell pellet.
5. Snap-freeze the crosslinked sample in liquid nitrogen and store at –80 °C.

NOTE

Prepare cells in a parallel culture dish under the same conditions to assess the cell number accurately for ChIP/HiChIP.

03 Adherent Cell Crosslinking

1. Discard the culture medium and add formaldehyde crosslinking buffer at a final concentration of 1%. For a 10 cm dish, use either 20 mL of 1× PBS containing 540 µL of 37% formaldehyde or 10 mL of 1× PBS containing 270 µL of 37% formaldehyde. Crosslink for 10 minutes at room temperature.
2. Prepare 0.125 M glycine using 1 mL of 10× PBS, 500 µL of 2.5 M glycine, and 8.5 mL H₂O.

3. After crosslinking, discard the crosslinking solution and wash with pre-chilled 1× PBS. Quench with 0.125 M glycine for 5 minutes at room temperature: use 10 mL for a 15 cm dish or 5 mL for a 10 cm dish.
4. Discard the glycine solution. Wash with 10 mL of pre-chilled 1× PBS and discard the PBS. Add 3–5 mL of pre-chilled 1× PBS on ice and scrape the cells from the dish with a cell scraper. Centrifuge at $500 \times g$ for 10 minutes at 4 °C, discard the supernatant, and retain the cell pellet.
5. Snap-freeze the crosslinked sample in liquid nitrogen and store at –80 °C.

NOTE

Prepare cells in a parallel culture dish under the same conditions to assess the cell number accurately for ChIP/HiChIP.

04 Tissue Crosslinking

1. Wash tissue containing residual blood with PBS. Use a knife to quickly cut the tissue into small pieces in PBS, with each piece no larger than 0.5 cubic centimeters. Add formaldehyde at a final concentration of 1%, using at least five times the tissue volume. Crosslink for 20 minutes at room temperature, then quench with glycine at a final concentration of 0.125 M.
2. If no further processing is planned, wash the crosslinked tissue twice with pre-chilled PBS and discard the wash liquid. Snap-freeze in liquid nitrogen and store at –80 °C for subsequent experiments.
3. If continuing processing, homogenize the tissue in pre-chilled PBS. Protease inhibitors are not required for this step. Filter through a 100 µm cell strainer into a 50 mL centrifuge tube and wash twice with 40 mL of pre-chilled PBS. Finally, freeze the tissue for subsequent sonication.

05 Recommended Number of Cells

ChIP-seq: 10 million cells per sample

HiChIP: 25 million cells per sample