

EPIGENOMICS SERVICES

Sample Preparation Guide for eCLIP-seq



SCOPE Animal Tissue Crosslinking · UV Crosslinking for Cells · Frozen Adherent Cells · Frozen Suspension Cells

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

01 Animal Tissue Crosslinking

1. Ensure each animal tissue sample is ≥ 200 mg.
2. Place an enamel tray into the crosslinking device.
 - In a 15 cm culture dish, prepare an ice–water bath by filling the dish with ice, leveling the ice, and adding water until the ice is just covered.
 - Cover the ice–water surface with a single, smooth layer of cling film (plastic wrap).
3. Fill a clean beaker with ice-cold PBS.
 - Using scissors, cut the tissue into small pieces (approximately 2 mm²) and immediately transfer the pieces into the PBS to keep them cold.
4. Remove tissue pieces from PBS and spread them in a single layer on top of the cling film in the culture dish.
 - Ensure tissue pieces are not overlapping or stacked and are in flat contact with the cling film.
 - Place the culture dish onto the tray inside the crosslinking device.
5. Set the crosslinker to 254 nm with an energy dose of 600 mJ/cm².
 - Run the first crosslinking cycle.
 - Allow samples to cool for 30 seconds. Check the ice–water bath and add ice as needed to maintain low temperature.
 - Run a second crosslinking cycle using the same settings (254 nm, 600 mJ/cm²).
6. Carefully blot the crosslinked tissue pieces using absorbent paper to remove excess moisture.
 - Transfer the tissue into labeled tubes approved by their manufacturer for the freezing procedure being used. Close the tubes according to the manufacturer's instructions. Freeze the samples using a procedure compatible with the selected tubes.
7. Store samples at -80 °C and ship on dry ice.

02 UV Crosslinking for Cells

1. Place an enamel (metal) tray into the UV crosslinker.
 - Prepare an ice–water bath in the tray: add ice, level the surface, then add water until the ice is just covered.

2. Sample processing:

- **a) Adherent cells.** Aspirate the culture medium. Wash the cells twice with ice-cold PBS, then remove PBS completely. Add ice-cold PBS to create a thin liquid film over the cell monolayer (approximately 1 mL per 10 cm dish).
 - **b) Suspension cells.** Centrifuge to pellet cells and discard the culture medium. Wash the pellet twice with ice-cold PBS, discarding the supernatant each time. Resuspend the cells in 1 mL pre-chilled PBS and spread evenly in a clean 10 cm culture dish.
3. Place the culture dish on the tray inside the UV crosslinker, ensuring stable, level placement.
 4. Set the crosslinker to 254 nm and 200 mJ/cm². Run one crosslinking cycle.
 5. Allow the samples to cool for 30 seconds. Check the ice-water bath; if most ice has melted, replenish with fresh ice to maintain low temperature.
 6. Using the same settings (254 nm, 200 mJ/cm²), run a second crosslinking cycle.
 7. Collect cells and transfer to a labeled 1.5 mL microcentrifuge tube. Centrifuge at 1,000 × g for 5 minutes at 4 °C, then discard the supernatant. Close the tube according to the manufacturer's instructions and freeze the cell pellet using a procedure compatible with the selected tube.
 8. Store samples at –80 °C and ship on dry ice.

03 Frozen Adherent Cells

1. Examine cells under a microscope to confirm healthy morphology and growth. A confluence of 80–90% is recommended.
2. Carefully aspirate the spent culture medium. Gently rinse the cells with pre-chilled 1× PBS, taking care to keep the monolayer intact and avoiding vigorous shaking. Repeat the PBS wash once.
3. Add a small volume of 1× PBS, then use a sterile cell scraper to detach cells. Transfer the suspension to a tube and centrifuge at 1,500 × g for 5 minutes at 4 °C. Discard the supernatant and transfer the cell pellet to a 1.5–2.0 mL cryovial (or microcentrifuge tube).
 - If needed, estimate pellet volume as a rough proxy for cell number: ~10 µL of packed cell pellet ≈ 1 × 10⁷ cells, approximately equivalent to the yield from a 10 cm dish at ~90% confluence. This estimate is based on HeLa cells and may vary with cell size and type; use as a reference only.
4. Freeze cells using a slow, controlled cooling method (e.g., a controlled-rate freezing container) and ship samples on dry ice.

04 Frozen Suspension Cells

1. Examine the cells under a microscope to confirm healthy morphology and growth. A healthy culture density is recommended for suspension cells.
2. Transfer the cell suspension into a 15 mL or 50 mL centrifuge tube. Centrifuge at 1,500 × g for 5 minutes at 4 °C. Discard the spent culture medium, then resuspend the pellet in pre-chilled 1× PBS to wash. Centrifuge again, discard the PBS wash, and repeat the PBS wash once.

3. Transfer the final cell pellet into a 1.5–2.0 mL cryovial (or microcentrifuge tube).
 - If needed, estimate pellet volume as a rough proxy for cell number: ~10 µL of packed cell pellet $\approx 1 \times 10^7$ cells, approximately equivalent to the yield from a 10 cm dish at ~90% confluence. This estimate is based on HeLa cells and may vary with cell size and type; use as a reference only.
4. Freeze the cells using a slow, controlled cooling method (e.g., a controlled-rate freezing container) and ship samples on dry ice.