

EPIGENOMICS SERVICES

Sample Preparation Guide for G4/G-loop/R-loop CUT&Tag

SCOPE Cryopreserved Cell Samples · Animal Tissue Samples

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

01 Cryopreserved Cell Samples

Cryopreserved cells can be shipped on dry ice. The safest approach for fresh cells is to cryopreserve them first and then ship on dry ice:

NOTE

During cell culture, contamination can occur due to mycoplasma, chlamydia, bacteria, fungi, or even cross-contamination between cell lines. Please note that CD Genomics' sample quality control does not include testing for these types of contamination. We therefore recommend implementing appropriate contamination-prevention measures throughout culture and ensuring that submitted cell samples are free of contamination.

1. Cell collection and washing.
 - **Suspension cells:** Harvest cultured cells, examine under a microscope to confirm healthy growth and absence of bacterial contamination, then wash with PBS and centrifuge twice to remove the supernatant (wash with 500 µL 1× PBS; centrifuge at 500 × g for 5 min at 4 °C). Proceed to cryopreservation using the cell pellet.
 - **Adherent cells:** Harvest cultured cells, examine under a microscope to confirm healthy growth and absence of bacterial contamination, gently rinse with PBS, remove PBS, and digest with an appropriate amount of trypsin. Add PBS to resuspend cells, collect by low-speed centrifugation, and discard the supernatant (wash with 500 µL 1× PBS; centrifuge at 500 × g for 5 min at 4 °C).
2. Resuspend the cell pellet in freezing medium and transfer to cryovials. Prepare freezing medium in advance and allow it to equilibrate to avoid overheating that could damage cells. Typically, CUT&Tag requires ~50,000 healthy cells (~1×10⁶ cells for G-loop) after thawing; therefore, we recommend freezing ~500,000 cells per sample.
 - **Freezing medium:** 60% complete culture medium + 30% FBS + 10% DMSO.
3. Place cryovials in a controlled-rate freezing container and store at –80 °C for stepwise cooling and temporary storage (strictly follow controlled-rate freezing procedures to minimize cell damage). If a controlled-rate freezing container is not available, use the following manual cooling protocol:
 - 4 °C for 10 min → –20 °C for 30 min → –80 °C for 16–18 h.
4. Transfer the cryopreserved cells from the controlled-rate freezing container (or from –80 °C) into liquid nitrogen for long-term storage, or ship on dry ice.

02 Animal Tissue Samples

1. Collect the fresh tissue. The recommended collection amount is >40 mg per sample (>80 mg for G-loop).
2. Transfer the tissue into a new centrifuge tube and rinse with pre-chilled PBS until all residual blood on the tissue surface is completely removed.
3. Gently blot off excess liquid with lint-free tissue. If the tissue piece is large, cut it into small portions on ice (approximately 40 mg per aliquot) and place each portion into a cryovial.
4. Immediately place the cryovials into liquid nitrogen for 30 minutes, then transfer to -80 °C for storage or ship directly on dry ice.