CUT&Tag-IT™* R-loop
Service Sample Preparation

Active Motif recommends preparing at least 400,000 cells for each CUT&Tag-IT™ R-loop reaction. If cells are limited, less may be acceptable with prior approval.

NOTE: Cells must be cryopreserved. Flash frozen cell pellets are not compatible with this service. For high quality data, we recommend sending samples with >70% cell viability. Please thaw a test sample to test post-thaw viability.

Reagents

Enzyme Free Cell Dissociation Solution Hank's Based (1X)- (Millipore-Sigma, Cat. No. S-004-M) or equivalent.

Cryopreservation of cells

1. Incubate Mr. Frosty or equivalent device at 4°C for a minimum of 1-hour prior to use.

2. For healthy adherent cells lines, use Enzyme-Free Cell Dissociation Solution and scrape cells with a rubber policeman or by pipetting. DO NOT use enzyme-based dissociation methods. For healthy suspension cells, transfer cells in growth media to a conical tube for pelleting.

3. Harvest cells at room temperature and count cells using hemocytometer or equivalent method to determine volume needed to achieve the proper concentration of cells for cryopreservation. While quantifying, keep cells on ice. Using low-bind microcentrifuge tubes may help avoid potential sample loss.

4. Centrifuge at 500 x *g* at 4°C to pellet the cells and remove supernatant.

5. Resuspend cells in 500 µL of ice-cold cryopreservation solution – 50% FBS/40% growth media/10% DMSO. Transfer 500 µL to a 1.5 mL Eppendorf tube on ice.

6. Freeze the cells by transferring the tubes to a pre-chilled Mr. Frosty container or equivalent device, like the one depicted below and place at -80° C.

7. If necessary, an alternate approach is to place the tubes upright in a styrofoam container. Close the styrofoam container with the styrofoam top and then place at -80° C.

8. Ship cells on dry ice.