

## scRNA-Seq Sample Preparation

We recommend preparing between **100,000 – 2,000,000 cryopreserved cells for scRNA-Seq**. Higher amounts of cells increase the chance of success. The cell preparation protocols below have been optimized for >2 million cells. If working with <500,000 cells, adjust wash step volumes and tube sizes (i.e. from 15ml conical to 1.5 mL Eppendorf tube) to minimize cell loss.

### I. Cryopreservation

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, enzymatically detach them using trypsin or another enzyme as needed for your specific cell type. For healthy suspension cells, transfer cells in growth media to a conical tube for pelleting.
3. Centrifuge at 500 x g at 4°C to pellet the cells and remove supernatant.
4. Resuspend cells in the appropriate volume of ice-cold cryopreservation solution – 50% FBS/40% growth media/10% DMSO – to achieve a concentration of 4 million cells/mL. If there are less than 2 million cells total, use 500 µL. Transfer 500 µL to a 1.5 mL Eppendorf tube on ice. Do not use screw cap or cryotubes that cannot fit into a microcentrifuge.
5. 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.



6. 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.
7. Ship cells on dry ice to Active Motif, 1914 Palomar Oaks Way, Ste 150, Carlsbad, CA 92008
8. Full shipping instructions can be found on our Sample Submission Form, which can be downloaded at the [scRNA-Seq Services web page](#).