

## FREEZING PROTOCOL

Always freeze down cells at a high concentration and at as low a passage number as possible. Ensure that the cells are at least 90% viable before freezing.

Always use proper aseptic technique and work in a laminar flow hood. Always wear personal protective equipment when working with liquid nitrogen.

1. Harvest log phase cells (with > 90% viability):

For adherent cells, gently detach the cells from the culture vessel to collect cells into a centrifuge tube following the Subculturing Protocol (step 1 to step 6).

For suspension cells, harvest all cells into a centrifuge tube.

2. Determine viable cell density and calculate the required volume of Cryopreservation Medium needed. We recommend freezing cells at  $1.0$  to  $2.5 \times 10^6$  cells/ml.
3. Centrifuge the cell suspension at 1500 rpm for 3 minutes.
4. Aseptically, aspirate out the supernatant without disturbing the pellet.
5. Re-suspend the cell pellet in Cryopreservation Medium at the appropriate cell density.
6. Dispense the cell suspension into cryovials and freeze according to your laboratory standard (i.e. controlled rate freezing at approximately  $1^\circ\text{C}$  decrease per minute).
7. Transfer the frozen cells into liquid nitrogen storage (in the gas phase above the liquid nitrogen) for long-term storage.

*For laboratory research only. Not for clinical applications.*

*For technical questions, please email us at [info@runtogen.com](mailto:info@runtogen.com)*