

## 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 ( $\underline{\text{TM024}}$ ). We recommend freezing cells at 1.0 to 2.5 x 10<sup>6</sup> 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°C decrease per minute).
- 7. Transfer the frozen cells into liquid nitrogen storage (<u>in the gas phase above the liquid nitrogen</u>) for long-term storage.

For laboratory research only. Not for clinical applications.

For technical questions, please email us at <a href="technical@abmgood.com">technical@abmgood.com</a>