CellBank Australia

Guideline for the resuscitation of cryopreserved cells

Cell lines frozen by CellBank Australia are shipped with a cell line datasheet which provides information regarding the Cell culture requirements of the particular cell line. Please consult the cell line datasheet before initiating your culture.

1. Turn on the UV light in the biohazard hood for 20 minutes. Prewarm and prepare the culture medium. Remove the vial from liquid nitrogen storage using appropriate safety equipment. Transfer the ampoule to the cell culture room on dry ice.

2. Remove the cryoflex wrapping from around each vial using small scissors and then thaw the vial quickly by immersion in a 37°C water bath. This will take no more than 1-2 minutes.

   Note: Rapid thawing in this manner is essential to minimize damage to the cells. If the cryoflex is not removed before thawing, the thawing will not be rapid and there is potential for contamination should water get in around the lid of the ampoule. Do not thaw ampoules in an incubator or in your hand.

   Immerse only the lower part of the vial in the water.
   Use 70% ethanol to wipe down the outside of the vial before transferring it to the biohazard hood (use a class 2 cabinet as a minimum).

3. Slowly pipette the whole contents of the vial into 9mls of pre-warmed growth medium in a sterile centrifuge tube.

4. Pellet the cells by centrifugation at 1,000 rpm for 5 minutes.

5. Remove the supernatant and resuspend the cells in 2-5mls of growth medium. Take a small aliquot of cells for counting.

6. Seed the cells into an appropriate size flask and place in the incubator at 37°C and 5% CO2.

   Ampoules frozen by CellBank Australia contain 2-4 million cells per vial and one vial can routinely be thawed into a T75 flask in 15mls of the appropriate cell culture medium.