



## Thawing Cryopreserved Cells

1. Defrost the vial in a 37°C water bath with constant, moderate agitation, until ice in the ampule is no longer visible.
2. Continue to warm the ampule in the water bath for 30 seconds with gentle agitation.
3. Immediately disinfect the vial with 70% ethanol.
4. Working in a hood, open the vial and transfer the contents to a sterile 15 ml tube. (Note: if thawing a glass vial, open the vial by wrapping the disinfected vial in a sterile gauze pad and breaking the neck of the pre-scored ampule).
5. Add 1.5 ml of culture medium (serum containing medium) that has been pre-warmed to 37°C.
6. Allow to stand for 5 minutes.
7. Add 3 ml of prewarmed culture medium and allow to stand for 5 minutes.
8. Add 6 ml of prewarmed culture medium and allow to stand for 5 minutes.
9. Centrifuge the suspended cells at 200 x g for 10 minutes.
10. Decant the medium and gently resuspend the cell pellet in 25 ml of culture medium and transfer into one 75 cm<sup>2</sup> culture flask.
11. Observe the cells microscopically to estimate cell viability and then place them in an incubator.
12. After overnight incubation, the cells should be observed so that the viability may again be evaluated. A trypan blue dye exclusion stain may be appropriate when precise viability assay is desired.