

Thawing MEF's:

Thomson Lab, 2004

1. Remove vial from liquid nitrogen.
2. Do a quick thaw in a 37°C water bath, being careful not to immerse the vial above the level of the cap.
3. When just a small crystal of ice remains, sterilize the outside of the vial with 95% EtOH and place it in the hood.
4. Gently pipet the cells up and down once, and place them in a 15 ml conical tube.
5. Dropwise, slowly add 10 ml culture medium to the tube. This should not take less than 2 minutes. To do this faster shocks the cells and you may end up with a lower viability than you otherwise would.
6. Spin the cells at 1000 rpm for 5 minutes.
7. Resuspend the cells in 10 ml culture medium and add to T75.
8. Place in 37°C incubator.
9. Fill out a freeze/thaw form to keep the database current.