

General Protocol: Thawing Cells

To Thaw Cells:

- ❖ Wear glasses and gloves before opening the liquid nitrogen tank
- ❖ Take the cell vial out from the liquid nitrogen tank, put the vial into an ice bucket immediately.
- ❖ Put 4 mls complete media into a new tissue culture T25 plate or P10 if densely frozen (collagen-coated).
- ❖ Place cells in a 37°C water bath, thaw as quickly as possible, less than 60 seconds.
- ❖ Transfer to waiting T25 flask
- ❖ The next day, replace all media with new complete media. Watch closely and expand to P10 or T25 when ready.
- ❖ Note: Growth rate will be slow after a freeze, and cell death always occurs in some fraction. Growing on a smaller surface will more readily allow cells to become healthy.