c) Freeze the Cells

1. Grow cells in a required flask. Sometimes it will be preferred to grow a large batch of cells preferentially in a T-75 flask.
2. Trypsinize each flask as described above.
    - T25 = 0.75 – 1.0ml trypsin
    - T75 = 1.5 – 2.0ml trypsin
3. Stop trypsinization with 2 (T25) or 4 (T75) ml of media.
4. Centrifuge at 1,000 rpm 4° C for 5-8 minutes.
5. Rule:
    - Most cells: 1,000 – 1,200 rpm
    - *Toxoplasma*: 3000 rpm for 8 min
6. Remove supernatant leaving as little volume as possible
7. Make the **freezing media** (see above)
8. Resuspend cells in desired volume of freezing media (~0.75 -1.0ml)
9. Aliquot into pre-cooled cryovials by placing tubes in the -20° C freezer.
10. Put cryovials in -20°C for an hour and move to the -80° C freezer.
11. 24 hours later, move to Liquid Nitrogen cryo storage and mark locations in the logbook provided. Try not to leave tubes in the -80° C freezer for more than 4 weeks.

d) Thaw and grow the cells

1. Remove a cryovial from Liquid Nitrogen storage (marking it off in the logbook). Put the vials in ice immediately.
2. Keep the vial in the rack under the hood. Agitate gently, until thawed.
   Note: Thawing should be as rapid as possible once the vial is removed from storage or keep the vial on the dry ice or cold until ready to finish.
3. Wipe the outside of the vial with 70% EtOH or the EtOH wipes, and uncap.
4. Remove cells with a sterile pipette, and place them in a cell culture flask with media.
5. Treat cells as normal passage. Label flask with all important information.