Cell Culture work

a) Media

DMEM Culture Media: 100 ml total

10 ml FCS (10%)
1 ml antibiotic/mycotic
1.5 ml Na Bicarbonate
1 ml Non essential aminoacids (0.1 mM)
1m l HEPES (10 mM)
67 ml DMEM

Freezing Media: 10 ml total

DMEM – 6.0 ml
FCS – 3.0 ml
DMSO – 1.ml

b) Split the cells

1. Start the biosafety cabinet and let it equilibrate for at least 3 min. Turn on UV light
2. Place the 1X trypsin into 37° C water bath for 3-5 min. Wipe it with 70% ethanol before using.
3. Label the new cell culture flasks with cell line type, passage number, date, and initials.
4. Make sure the cells are confluent, (complete growth of monolayer) and that there are no signs of contamination.
5. Remove the culture media from flask using sterile pipette and dispose the media into waste beaker container.
6. Add 1X trypsin
    a. T25 flask - ~ 0.75 - 10 ml trypsin and swirl flask (do not bring media into the neck).
    b. T75 flask - ~ 1.75 - 2.0 ml of 1X trypsin for T75 flask.
    - Remove inactivated trypsin. Add trypsin a second time and observe under the microscope
    - The first trypsin volume removes the serum and remaining media. The second volume works to detach cells.
7. Quickly mix flask in order to cover entire cell monolayer with trypsin. Tapping on the bottom or side of the flask will help release any adhered cells.
8. Place flask on the microscope and check for cell detachment.
9. Add approximately a 1:2 ratio of media to the flask to stop the reaction. (trypsin:media)
10. Use pipette to draw up and expel the media onto the cells. (This step forces the adherent cells to be released and can also brake clumps into single cell suspension). ****Note**: Be

careful about splashing media onto sides of flask, as this will increase the amount of debris in the flask.** Do not splash media into the neck!!

11. Aliquot media + cell solution into number of flasks as needed.
    a. Next 2 days → add 0.75 – 1.5 ml
    b. Within a week → add 0.25 ml
12. T25 flask received a total of **3.0 – 3.5** ml (cells + media volume needed) while a T-25 flask = **9.0 - 10** ml
11. The flask used in the split receives fresh media and is placed back in the incubator. Incubate flasks at 37° C.
12. Record split and passage number in calendar or lab book.
13. If needed, change media in flask after 24 hours if dead cell debris is significant.
14. Keep track of the passage number each time you split the cells. When cells reach P25, thaw a new tube batch to expand.

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 8 minutes.
   - Rule: 1,000 – 1,200 rpm for most cells
   - *Toxoplasma*: 3000 rpm for 8 min
5. Remove supernant leaving as little volume as possible
6. Make the **freezing media** (see above)
7. Resuspend cells in desired volume of freezing media (~0.75 -1.0ml)
8. Aliquot into pre-cooled (-20° C) cryovials. Leave the tubes on ice (or freezer) until you fill all the freezing tubes with cells.
9. Put cryovials in -20°C for an hour and move to the -80° C freezer.
10. 24 hours later, move to Liquid Nitrogen cryo storage and mark locations in the logbook provided.

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 rapid as possible once the vial is removed 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 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.