Cell Culture work

a) Media

DMEM Culture Media: 50 ml total

5 ml FCS (10%)
0.5 ml antibiotic/mycotic
0.75 ml Na Bicarbonate
0.5 ml Non essential aminoacids (0.1 mM)
0.5 ml HEPES (10 mM)
make up to the 50 ml line with DMEM

Freezing Media: 10 ml total

DMEM – 6.25 ml from above
FCS – 3.0 ml
DMSO – 0.75ml

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.
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 a sterile pipette and dispose the media into waste beaker container.
6. Add 1X trypsin and swirl carefully to make sure the trypsin comes in contact with the whole cell surface
    a. T25 flask - ~ 0.75 – 1.0 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.

7. Remove inactivated trypsin.
8. 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.
9. 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.
10. Place flask on the microscope and check for cell detachment.
11. Add approximately a 1:2 ratio of media to the flask to stop the reaction (trypsin:media).
12. Use pipette to draw up and expel the media onto the side of the flask. (This step forces the adherent cell layer off the side of the flask. It also helps in preventing by chunks of cells).

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!!

13. Aliquot media + cell solution into number of flasks as needed.
14. T25 flask received a total of **3.0 – 3.5** ml (cells + media volume needed) while a T-75 flask = **9.0 - 10** ml
15. Unless you do not have a need, in most cases the flask used in the split receives fresh media and is placed back in the incubator. Incubate flasks at 37° C.
16. Record split and passage number in your lab book.
17. If needed, change media in flask after 24 hours if dead cell debris is significant.
18. Keep track of the passage number each time you split the cells. When cells reach P20, thaw out a new tube batch to expand.