Establishing a HeLa Cell Line to Implement Protocols for NHTI Cell Biology Labs

By Patrick Jones, Beth Wilkes, Karel Pluhar and Benjamin Moyer

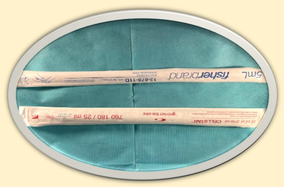
Introduction / Materials

Hands-on learning is one of the best ways for student to not only get better at a subject and the practical uses for it, but it's also a way for student to gain an interest in something they may not have had before.(2)

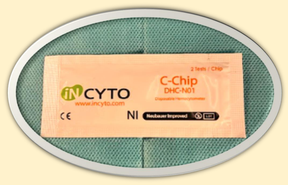
The establishment of a mammalian cell line, and the protocols to maintain it will be developed for the cell biology class at NHTI. This will give the students the opportunity to work with an established cell line.

The following images show the materials used to sterilize all equipment and work surfaces as well as establish a HeLa cell line.

Sterile pipets



Hemocytometer

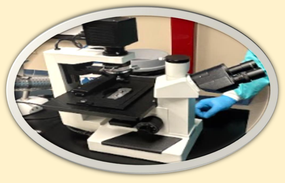


T25 flasks

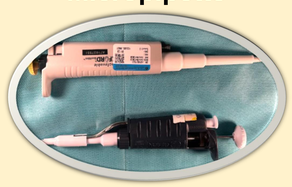
Handheld counter

70% ethanol/ Paper towels

Microscope



Micropipette



Suspension media

Procedures

Figure 1: Ready ethanol wipes

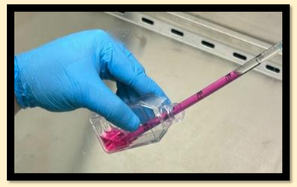


Figure 4: Multiple pipetting steps including draining media to applying PBS and Trypsin

Figure 7: Centrifuge to separate trypsin from cells

Figure 2: Start with the top and back surfaces

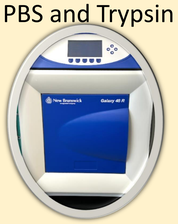


Figure 5: Incubate to warm trypsin helping the enzymatic reaction

Figure 8: Empty supernatant after centrifuge

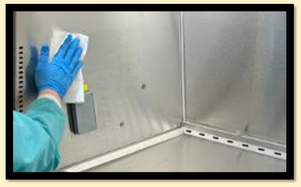


Figure 3: Wipe the side and working surfaces.

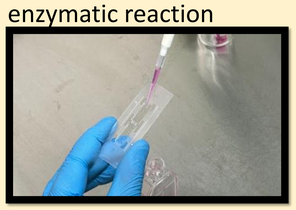


Figure 6: Micropipetting dislodged cells into hemocytometer

Figure 9: Resuspend, then micropipette desired concentration

Suspension Media

Suspension media provides vitamins, minerals and aminos acids for the cell nourishment

DMEM

Dulbecco's modified eagle medium

Basal media, makes up about 90% of suspension media

FBS

10% Fetal Bovine Serum

Universal growth supplement

P/S/F

Penicillin/Streptomycin/Fungicide

Anti-Bacterial and Anti-Fungal

1:100 of the volume of overall media

CHO and HeLa Cells

Mammalian cells

Commonly used in science and medicine

Grown in suspension media(1)

Special Materials

PBS

Phosphate buffer saline

Helps wash away excess media after draining it.

Trypsin

Digestive Enzyme

Breaks down proteins that help adhere cells to the back of the flask to dislodge them.

Incubator

Warms trypsin during counting stage to speed up detachment. Cells kept here in 5% CO_2 for pH regulation.(3) CO_2 depletion resulted in use of HEPES buffer.

Centrifuge

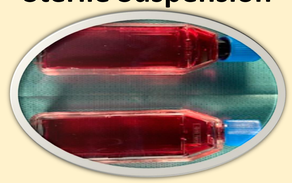
To remove trypsin from the cells.

HEPES

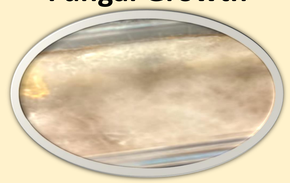
pH buffer used when CO_2 is not available to maintain healthy pH for cell survival and growth.

Problems

Sterile Suspension



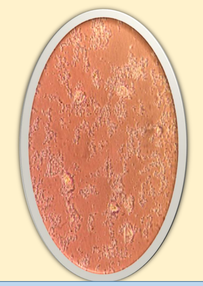
Fungal Growth



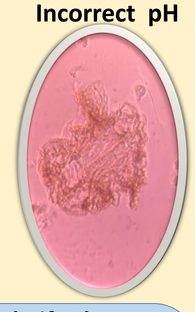
Bacterial and fungal contamination can happen for a multitude of reasons during an experiment. Ensuring that all solutions, buffers, beakers, and flasks used in the experiment are sterile is essential. Overall using PPE and making sure to sterilize correctly is extremely important.

Healthy CHO Cells

CO_2 Deprived CHO Cells



HeLa Cells After Incorrect pH



HeLa cells can be irreversibly damaged if they are maintained at the incorrect pH for any prolonged period. Both CHO and HeLa cells thrive at a pH of 7.3-7.9. (1) When running out of CO_2 in the incubator caused cell death, an attempt was made to use HEPES in our cell suspension to reestablish the correct pH. Unfortunately, the pH level ended up at a pH of 9 or above, leading to further cell death.

Acknowledgement

CBEC- The Cell Biology Consortium gave a lot of help to start this process and continue to help students learn hands-on protocols and start cell lines for their respective schools.

UNH Manchester- UNH Manchester deserves a massive thank you for supplying us with CHO and HeLa cells throughout the experiment.

The NSF funded RCN: UBE grant helped with the experiment by giving us money to attain the supplies needed for cell splitting and creating a cell line.

References

1-Rahbari, R., Sheahan, T., Modes, V., Collier, P., Macfarlane, C., & Badge, R. (2009, April 4).*A novel L1 retrotransposon marker for HeLa cell line identification*. National Library of Medicine National Center for Biotechnology Information. Retrieved February 25, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2696096/

2-Schwichow, M., Zimmerman, C., Crocker, S., & Hartig, H. (2016, February 24). *What students learn from hands-on activities*. Wiley Online Library. Retrieved February 4, 2023, from https://onlinelibrary.wiley.com/doi/10.1002/tea.21320

3-Dumont, J., Euwart, D., Mei, B., Estes, S., & Kshirsagar, R. (2015, September 18). *Human cell lines for biopharmaceutical manufacturing: history, status, and future perspectives*. National Library of Medicine National Center for Biotechnology Information. Retrieved February 13, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5152558/