**Cell Culture Primer**

*Walker Orr, 08/21/2023*

**General Principles**

Sterility: avoiding contamination is paramount in cell culture and we take several steps to ensure this sterility.

First set up three “zones” in your hood so you work in one direction, from clean to dirty. On the left is your “clean area”, where clean supplies (tips, tubes, etc). go. In the middle is your “working area,” where you’ll place and work with your flasks of cells. On the right is your “waste area” where the trash container will go. Cross this only from left to right so clean items stay clean and dirty items dirty.

Move slowly in the hood, as rapid movements can disrupt the sterilizing action of the laminar flow and create air currents that carry contaminants within the hood.

Everything that comes into the hood should be sprayed with ethanol to disinfect it, especially your gloves and the media bottles. Nathania does not spray plastic bags and other consumable packaging; Patrick does.

Cell culture flasks have a large plastic base to which cells adhere, and a closed narrow aperture at the top. Work with flasks upright but don’t leave them upright while cells are adhered to the plastic, as this can dry them out. Always recap flasks immediately. Use one hand to open the flask and hold the cap in that hand while you’re working in the flask, then replace it immediately when you are finished.

Use dedicated bottles of reagents for each cell type that is being used, to prevent cross-contamination.

**Set Up:**

37C DMEM, with FBS and Pen/Strep\*

37C Dulbecco’s PBS\*

TrypLE (room temperature)

*\*Make sure to start these items warming in the water bath for 15-20 minutes before use.*

**Passaging Cells**

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| **Steps** |  | **Helpful Hints** |
| 1. Estimate % confluency by examining cells under a microscope ( X mag) |  | Note the color of the media, it will yellow as it accumulates acid from the metabolic action of the cells. |
| 1. Use a Pasteur pipette to remove used media from the cells. |  | Attach via the wider glass bulb so you’re not handling the skinny portion. |
| 1. Add enough Dulbecco’s PBS to cover the monolayer of cells. Gently spread the PBS by rocking to wash the cells. Remove PBS with a Pasteur pipette. |  | 5-mL for a T75  7-mL for a T75 |
| 1. Add TrypLE. Transfer to 37C incubator for a few minutes to release cells from the flask. Visually examine cells to make sure they’re released; if they need help, give the side of the flask a gentle “thump” to release them. |  | 3-mL for a T75  6-mL for a T175 |
| 1. Add 37C DMEM. Resuspend well to break up clumps by pipetting repeatedly against the side of the flask, but do not introduce air bubbles. |  | 3 mL T75; 6 mL T175.  After the cells are resuspended, it’s okay to leave the flasks upright. |
| 1. Count the cells. Prepare a microcentrifuge tubes. Add 30 ul Trypan Blue to 30 ul of cell sample. Mix and add 10 ul of the mixture to the Countess slide. Record the number of live cells per mL. >90% live is the target. |  | Do not use a micropipette directly inside the cell flask. First, transfer a small volume of cells to a clean microcentrifuge tube using a serological pipette. Then use this stock for counting. |
| 1. Pass the cells to a new flask, or remove enough media from the current flask to achieve the desired seeding density. Passing at 1:5 (i.e., using 1/5 of the resuspended cells to seed a new flask, or removing 4/5 of the resuspended cells in the same flask) is typical. Gently rock the cells to distribute them against the bottom of the flask. Add fresh DMEM at the recommended volume. |  | 15 mL for a T75  35 mL for a T175 |
| 1. Label flasks with ethanol-resistant laboratory marker. Name, date, number of cells seeded (or passing ratio), number of passages. |  | After 20 passages, it’s time to thaw a new cell line. |
| 1. Return flasks to the incubator. They will adhere as they rest without disturbance. |  |  |
| 1. Clean the hood. Aspirate unused reagents to liquid waste. Clean the work area with bleach or microchem, then spray with ethanol and wipe down. |  |  |

**Virus Work Supplement**

* Microchem and ethanol the hood before and after every use.
  + Also thoroughly clean the aspirator and tubing with microchem and ethanol
* Take your time, do not be stressed! Do not rush.
* Mistakes will happen.
  + If you spill on your gloves, remove them carefully and wash hands thoroughly.
  + If you spill in the hood: microchem immediately. Let stand and wipe in place. Ethanol, and wipe again.
  + If exposure/cuts: Contact OMS.
* Adam maintains the hood aspirator trap.
* When you are finished: microchem the shit out of the hood.