## Week 1: Media Preparation

**Date: Jan 18 2023**

## Objectives, Hypotheses

Prepare the culture media that we’re going to use during the rest of the semester

* Practice sterile technique
* Proliferation Media Formulation: DMEM + 10% FBS + Primocin + 2 ng/ml FGFb

Also prepare solutions for the primary cell isolation next week

* DPBS with 10X Anti/Anti

## Materials Required

Dulbecco's phosphate-buffered saline (DPBS)

100X Antibiotic/Antimyotic

DMEM

Fetal Bovine Serum (FBS)

500X Primocin (antimicrobial)

Fibroblast growth factor beta (FGFb)

## Protocol/Procedure

Sterile Technique Pointers

* Anything that goes into the biohood (including your gloved hands) should be sterilized with 70% ethanol
* Don’t block biohood vents with pipette tip boxes, waste boxes, tube racks, etc
* Don’t allow liquid to get into the pipette guns/micropipettes
* Don’t invert the serological pipette when any liquid is inside, otherwise liquid will flow towards the pipette gun/micropipette and can affect the pipette filter
* Later with cells, never spray liquid directly onto the cells (i.e the bottom surface of the flask/well plate). Dispense along the side of well/flask.

DPBS + Anti/Anti

1. Take 90 ml of DPBS and add to a 150 ml bottle using a serological pipette
2. Add 10 ml of 100X Anti/Anti using a serological pipette
	1. Make sure that the anti/anti is homogenous if it was just thawed by pipetting up and down to mix
3. Store at 4C with your group name + the date + the class

Proliferation Media

1. Add 17.5 ml of DMEM to a 500 ml bottle of DMEM using a serological pipette.
2. Add 57.5 ml of FBS to the DMEM bottle using a serological pipette.
3. Add 1.15 ml of Primocin to the DMEM bottle using a micropipette
4. Add 11.5 ul of 0.1 mg/ml FGFb to the bottle
5. Store at 4C with your group name + the date + the class

## Data/Observations/Conclusions