**Seeding cells in the imaging plates using the Multidrop Combi**

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This protocol describes the procedure to dispense 40 ul of trypsinized cells suspension into the wells of an imaging plate.

**Equipment used:**

* ThermoFisher Multidrop Combi dispenser (Room 41/D304, cell culture hood)
* ThermoFisher Standard tube dispensing cassette (Cat# 24072671)

**Reagents:**

* 40 ml of 10% v/v bleach in a 50 ml conical tube.
* 2 X 40 ml of sterile water in 2 separate 50 ml conical tubes.
* 40 ml of 0.1% v/v Tween 20 in water in a 50 ml conical tube.
  + Add 40 ul Tween 20 to 40 ml water.
* 2 x 40 ml of sterile PBS in a 50 ml conical tube.
* Complete cell culture medium with 2X FBS.
* OPTIMEM serum-free medium.

**Cells:**

* At least 40 ml of cells resuspended in OPTI-MEM (Medium + 2X FBS) at the appropriate concentration in a 50 ml conical tube. For cell density testing:
  + Calculate 15 ml for initial priming, then 20 ml volume for an ENTIRE plate.
  + Add 40 ul per well.
* Count cells using an automated cell counting instrument. Always use the same instrument for downstream applications. The HiTIF is equipped with the BioRad TC20 Automated Cell Counter.

**Tips and Tricks:**

* Do NOT run the tubing dry between solution changes. It is our experience that bubbles form at the nozzles and interferes with volume dispensing and sterility.
* Do NOT allow salted solutions (PBS, medium, etc) to “sit” in dispenser. Evaporation will result in salts clogging the small bored nozzles.
* Follow the solution sequence as specified with priming volumes.
* Beware of the BLACK cords. If these wrap around the peristaltic pump wheel, you **cannot** attach the cassette. Ensure these are **outside** of the peristaltic pump wheels. This is a very common problem.
* Submerge the end of the dispensing tubes to the bottom of the tube.
* Be mindful of where you grab the tubing as you aseptically sterilize your tubing.

**Preliminary Steps:**

* Prepare the solutions to sterilize the tubing.
* Use the keypad on the Multidrop to have the correct program displayed and ready to go.

**Using the Multidrop in 4 parts:**

**PART 1: Harvest cells from your flask(s).**

1. Centrifuge to pellet cells. (1,500 RPM for 5 minutes).
2. Resuspend cells in 10 ml of complete medium.
3. Conduct a cell count using the Auto Cell Counter.
4. Resuspend cells at the appropriate cell density in 40 ml of complete medium.

* Final volume of 40 ml per Tube. The number of tubes is dependent on the total number of plates you are transfecting.
  + - The first 40ml is for **priming** and for the **first plate**.
    - The next 40 ml are for 2 plates. I do not try to squeeze a 3rd plate.
* Prepare an extra tube of cells, just in case.
  + Some indicative cell numbers:
    - 600 cells/40 ul per well 🡪 1.5x 104 cells/ml
    - 1,000 cells/40 ul per well 🡪 2.5 x 104 cells/ml
    - 1,500 cells/40 ul per well 🡪 3.75 x 104 cells/ml
    - 2,000 cells/40 ul per well 🡪 5 x 104 cells/ml
    - 2,500 cell/40 ul per well 🡪 6.25 x 104 cells/ml

**Prepare the Multidrop by sterilizing the cassette tubing.**

1. Wear gloves.
2. Mount the dispensing cassette on the MultiDrop.
3. Remember to pull the blue shield all the way forward.
4. Connect the drain tube to the aspirator and turn on the vacuum.
5. Submerge the aspiration end of the cassette tubes in the bleach 10% tube, all the way to the bottom of the tube.
6. Prime the cassette with 20 ml of bleach 10%. To prime, press the PRIME button on the keypad.
7. Prime with 20 ml of water (Tube 1).
8. **IMPORTANT: change the water tube!**
9. Prime with 20 ml of water (Tube 2).

**PART 3: Dispense cell suspension to your plate.**

1. Prime the cassette with 20 ml of PBS.
2. Add your plate to the stage.
3. IMPORTANT: remove the plate lid!
4. Vortex your cells to mix.
5. Dispense 40 ul of cell suspension to each well. To dispense the 40 ul, press START on the keypad.
6. Place the plate lid back onto the plate.
7. Process the next plate as needed (i.e. repeat steps 11 to 15 included).
8. Incubate plate(s) at RT for 30 minutes. (With lid on and in the hood). Continue immediately to the next step.

**PART 4: Decontaminate your cassette**

1. Prime the cassette with 20 ml of PBS.
2. Prime the cassette with 20 ml of 0.1 Tween 20%.
3. Prime the cassette with 20 ml of bleach 10%.
4. Prime with 20 ml of water.
5. **IMPORTANT: change the water tube!**
6. Prime with 20 ml of water.
7. Prime the cassette dry.
8. Slide the blue shield back.
9. Remove and place the cassette back in the box.
10. Write down the estimated total volume used in the experiment (Including all washes)