24 Well-Plate Stimulation

1. Each well should have:
   - 50,000 cells/mL
   - Volume: 1 mL

2. Before embarking on your stimulations, plate the 16 wells shown with arrows with the above cell numbers
   - Label the cover of the plate as shown above to keep track of your stimulations
   - Note: The last column is negative control which is essentially complete medium (16 wells with cells + 4 wells with medium only = 20 wells)

3. Use dilution formula:
   - Let’s assume your stock cell concentration is $7 \times 10^6$ cells/mL and you will be preparing 18 mL (always prepare some extra) of cell suspension at a final cell concentration of $1 \times 10^6$ cells/mL

   \[ C_1V_1 = C_2V_2 \]
   \[ V_1 = \frac{(0.050 \times 18 \text{ mL})}{7} = 0.128 \text{ mL} \]
   i.e Mix 0.128 mL stock cell suspension with 17.872 mL complete medium.
   Total is 18 mL

4. Aliquot 1 mL in each well (use 1 mL pipettor, accuracy is important)
   - Shake tube continuously to avoid cell precipitation at the bottom of tube
|   | Proceed with LPS stimulation at 100 ng/mL. Use dilution formula, you will be given the LPS stock concentration  
|   | Example: if stock concentration is 1 mg/mL it means 1000 ng/µL  
|   | Use dilution formula: \( C_1 V_1 = C_2 V_2 \)  
|   | \( V_1 = \frac{C_2 V_2}{C_1} = \frac{(100 \text{ ng/mL} \times 1000 \text{ µL})}{1000 \text{ ng/µL}} \)  
|   | Note the units of \( C_2 \) and \( C_1 \) are not the same, multiply denominator with 1000 µL/mL to change  
|   | \( V_1 = 0.1 \muL \), this is smaller than our P2 pipettor can handle  
|   |   | Dilute x100 the LPS stock concentration  
|   |   | You will end up needing 10 µL per well after x100 dilution  
|   |   | This is a reasonable volume  
|   | Aliquot 10 µL (use P20 pipettor) in each of the wells designated on the diagrams as 100 ng/mL  
|   |   | For 10 ng/mL wells, you do not need to recalculate  
|   |   | Simply make a x10 dilution of the previous dilution  
|   |   | Make sure you end up with enough for at least 6 wells  
|   |   | Example: Use 10 µL + 90 µL medium = 100 µL total (you will only end up using 60 µL for the 6 wells)  
|   |   | Aliquot 10 µL per well  
|   | Repeat for 1 ng/mL as above  
|   | Negative Control is just the medium you use for culturing cells. You should not be detecting anything in the supernatants of this group  
|   | Cover plate and place in the CO\( _2 \) incubator  
|   | Collect supernatants in 24 hrs.  
|   | Note: Some proteins take longer, some shorter  
| Before collecting supernatants:  
|   | Label lids (use thin tip marker) of 1.5 mL microcentrifuge tubes and place in a rack  
| Tilt plate 45 degrees and using the 1 mL pipettor, carefully remove ~800 µL supernatant  
| Change tips when moving to a different treatment  
|   | Save supernatants in −80 degrees Celsius freezer for long term storage  