1. Culture the plate with the indicated cell concentrations

2. You will be preparing 4 different tubes with the indicated concentrations
   - Each tube will have 2,000 μL, sufficient for the 8 wells you are plating

3. Use dilution formula:
   - Let’s assume your stock cell concentration is 3 x 10^6 cells/mL and you are preparing 2,000 μL (always prepare some extra) of cell suspension at a final cell concentration of 50,000 cells/mL

\[ C_1V_1 = C_2V_2 \]
\[ V_1 = (0.050 \times 2,000 \text{ μL}) / 3 = 33.3 \text{ μL} \]
- i.e. Mix 33.3 μL stock cell suspension with 1,963.7 μL complete medium.
- Total is 2,000 μL
   - Repeat for the remaining 3 cell concentrations

4. Aliquot 200 μL in each well (use P200 pipettor, accuracy is important)
   - Shake tube continuously to avoid cell precipitation at the bottom of tube