## Overview of Module 5

1. What is one reason we need to count cells?

2. What is the primary, manual method of cell counting used in research?

3. Why do we use hemocytometers?

4. In flow cytometry, what does forward scattered light help measure? What does side scattered light help measure?

5. In FACS, how are different cell types sorted?

6. Why is it important that a Coulter Counter contains electrolytic solution?

7. Why do we perform Viability Assays?

8. What is the function of mitochondrial reductase in an MTT Assay?

9. What is the function of luciferase in Cell Titer Glo?

10. What is the function of esterase in Live-Dead Assay?

---

**Cell Counting Techniques Vocabulary**

1. Match the key term to its role in cell counting or viability assays:

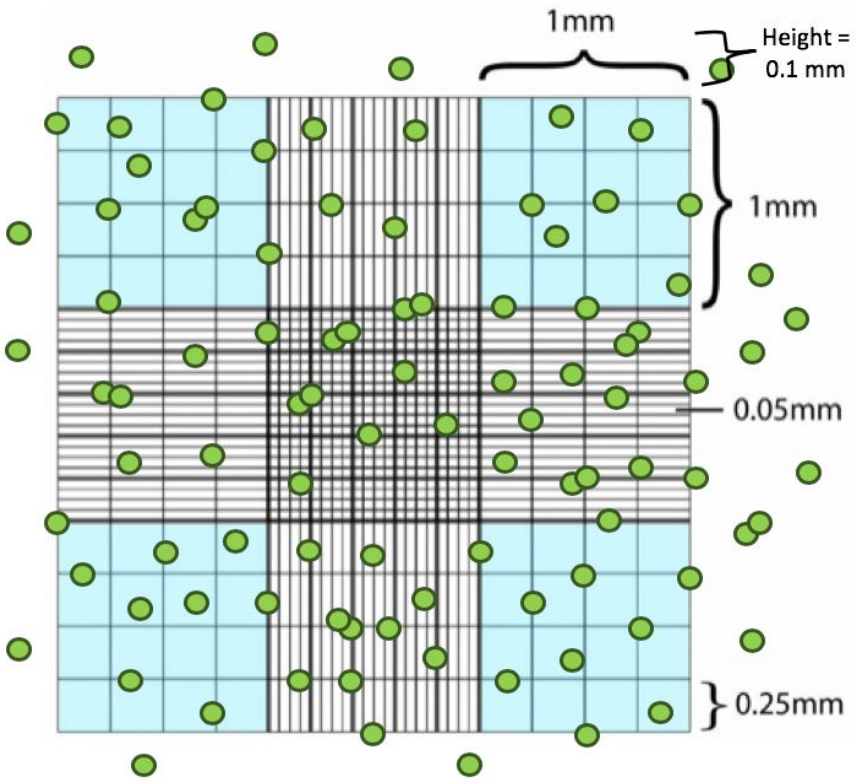
| | | |
|---|---|---|
| A. Calcein | _____ | Assay that uses bioluminescence to measure ATP |
| B. MTT Assay | _____ | One-step process that estimates metabolic activity through Formazan |
| C. Flow Cytometry | _____ | Two chamber cell counting system that measures changes in resistance |
| D. DMSO | _____ | Measures cells by detecting scattered light |
| E. ImageJ | _____ | The first modern cell viability assay |
| F. Hemocytometer | _____ | Common image analysis software for cell counting |
| G. Cell Titer Glo | _____ | Marks live cells in Live/Dead Assay |
| H. MTS Assay | _____ | Marks dead cells in Live/Dead Assay |
| I. Ethidium Homodimer | _____ | Manual cell counting method that uses counting grids |
| J. Coulter Counter | _____ | Reagent that creates pores in the cell membrane |

## Using a Hemocytometer

1. To ensure consistency in your hemocytometer count, you include cells on the _____ edge and _____ edge of your quadrant in your cell count, but don't include cells on the _____ edge and _____ edge of your quadrant in your cell count.

2. To obtain the average number of cells per counting square, you count the cells in each counting square and divide by _____.

3. To obtain the cell concentration in # cells / mL, you take the average number of cells per counting square, divide by the volume of each counting square ( ___ $mm^3$ ) and convert $mm^3$ to mL.
4. Practice obtaining a hemocytometer count!




**Quadrant Counts**

Square 1: _____ cells

Square 2: _____ cells

Square 3: _____ cells

Square 4: _____ cells

Average: _____ cells/square

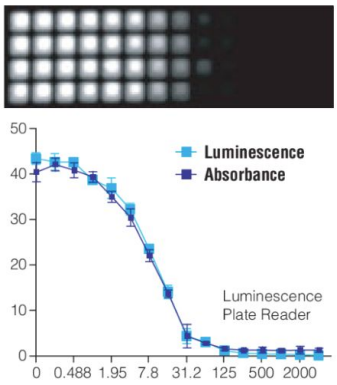
Cell Concentration:

$$\frac{cells}{mL} = (\ \_\_\_\_\_\ \frac{cells}{square})*(\frac{counting\ square}{0.1\ mm^3})*(\frac{1\ mm^3}{10^{-3}cm^3})*(\frac{1\ cm^3}{1\ mL}) = \_\_\_\_\_\ \frac{cells}{mL}$$

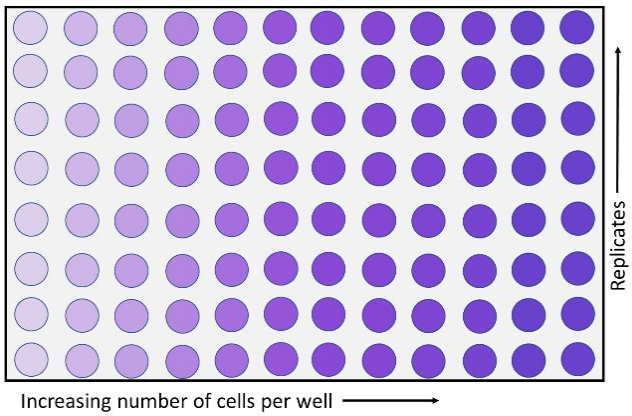
## Examining Cell Viability

5. Match the cell viability assay to the image below:

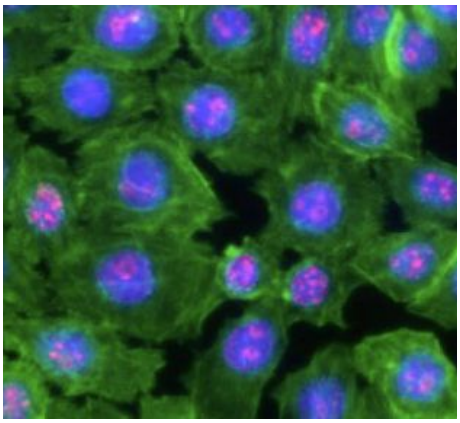
MTT/MTS, Live/Dead, Immunostaining, Cell Titer Glo



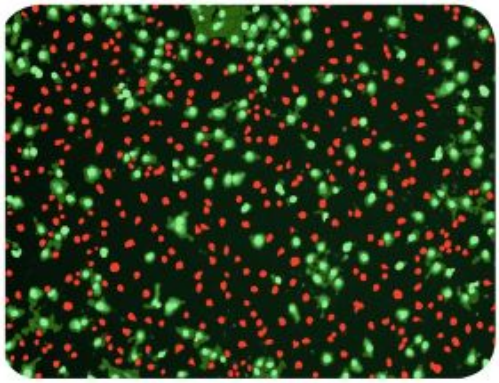

(A)




(B)



(C)



(D)

(a) Picture A shows the results of a _____ assay.

(b) Picture B shows the results of a _____ assay.

(c) Picture C shows the microscopy images from a _____ assay.

(d) Picture D shows the microscopy images from a _____ assay.