

Cell Proliferation

CELL PROLIFERATION

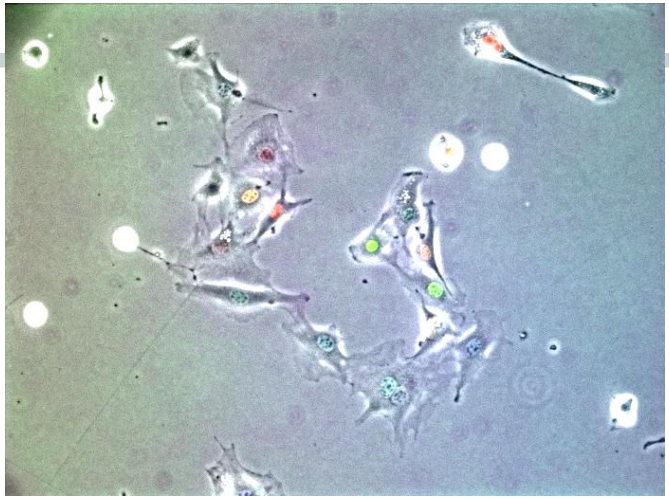
• Measurements of cell proliferation are often used to determine the response of cells to a particular stimulus or toxin.

• Also very important in routine maintenance as it is critical for knowing the best time for subculture, the optimum dilution, and the effects of changes in medium or cell culture vessels. ***Helps you to get to know your cells.***

• Knowledge of the growth state of a culture is critical in the design of cell culture experiments.

e.g. As cells reach the plateau phase, cell proliferation decreases. This could alter perception of the 50% inhibitory point (IC_{50}) in a toxicity experiment.

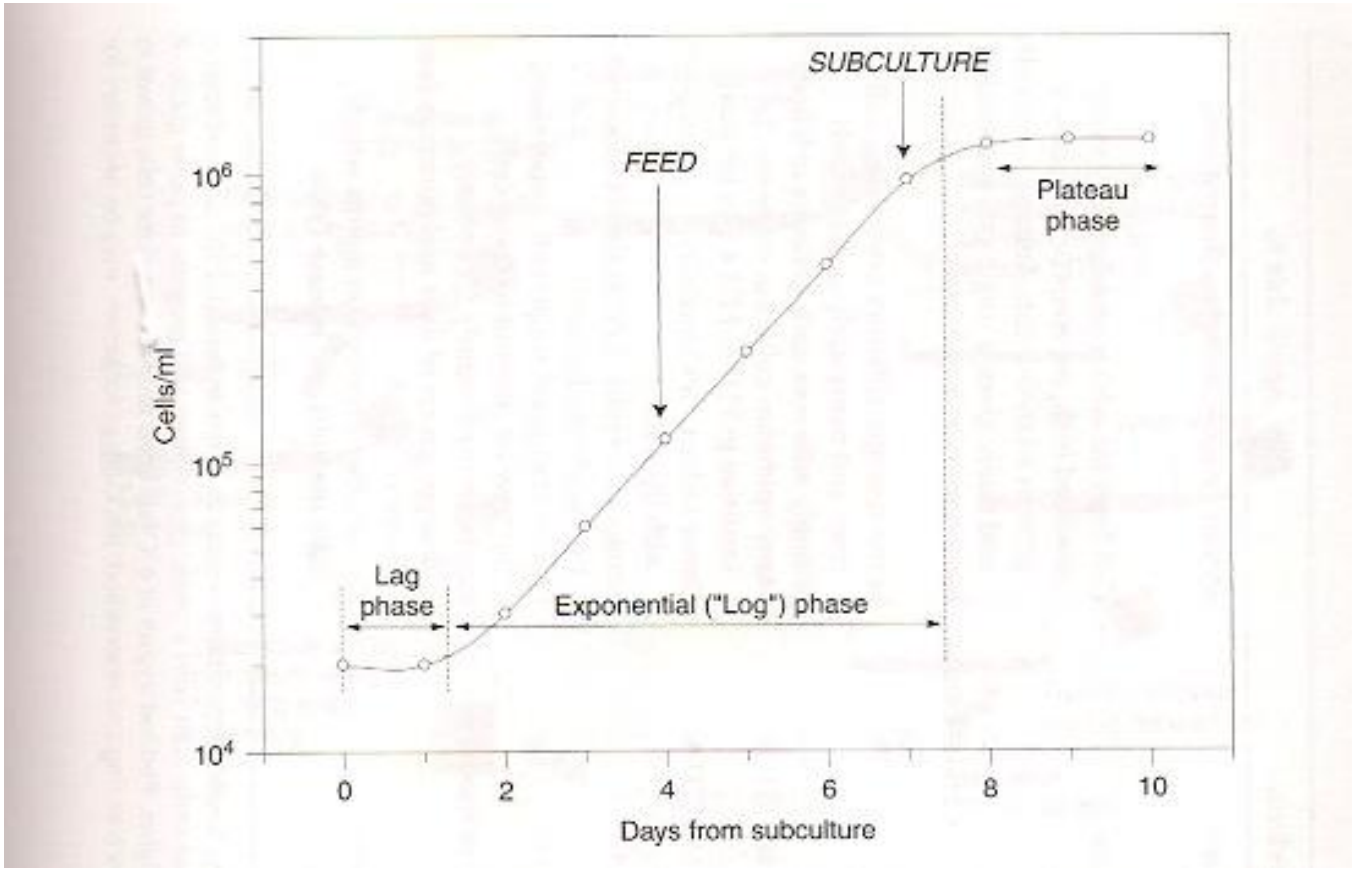
MEASUREMENTS OF CELL PROLIFERATION



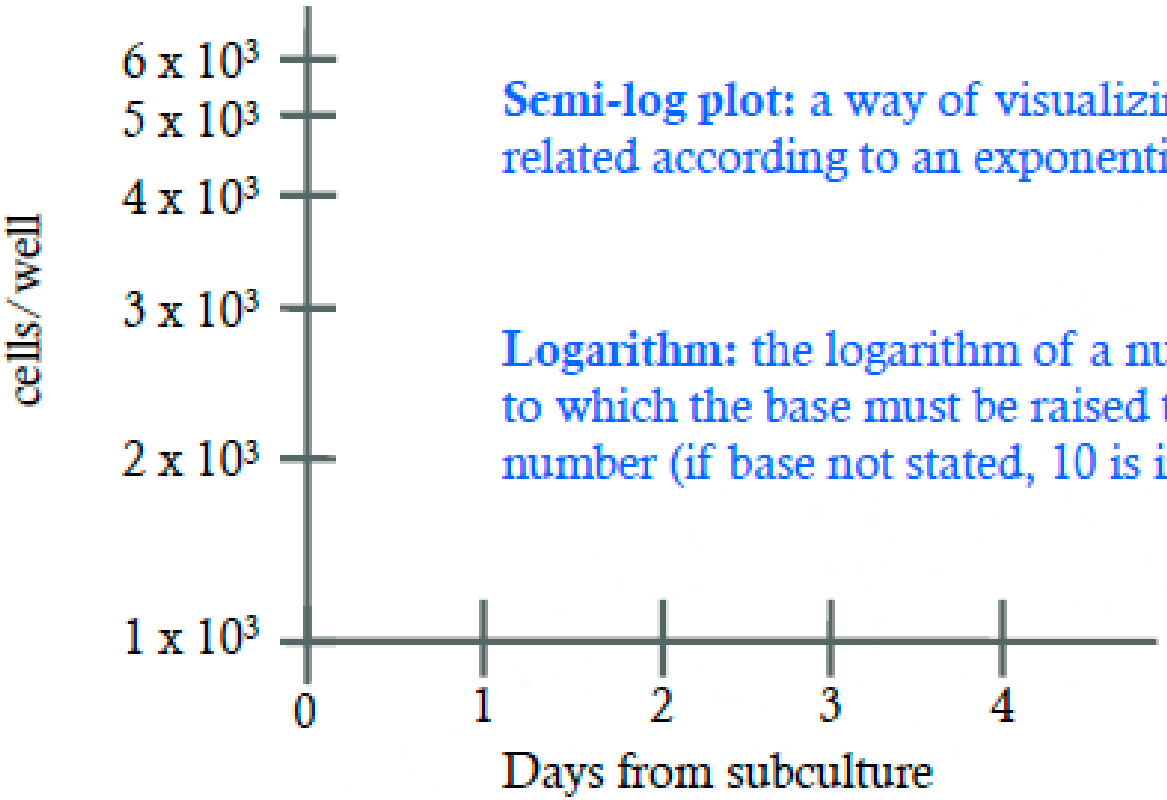
Population Doubling Time: Time for a given cell population to double. Product of cell division, cell death, and non-dividing (senescent) cells.

Growth Curve: Semi-log plot of cell number vs. time from subculture. The growth curve is used to determine population doubling time.

GROWTH CURVE



Semi-Log Plot for Growth Curve



Semi-log plot: a way of visualizing data that are related according to an exponential relationship

Logarithm: the logarithm of a number is the exponent to which the base must be raised to produce that number (if base not stated, 10 is implied)

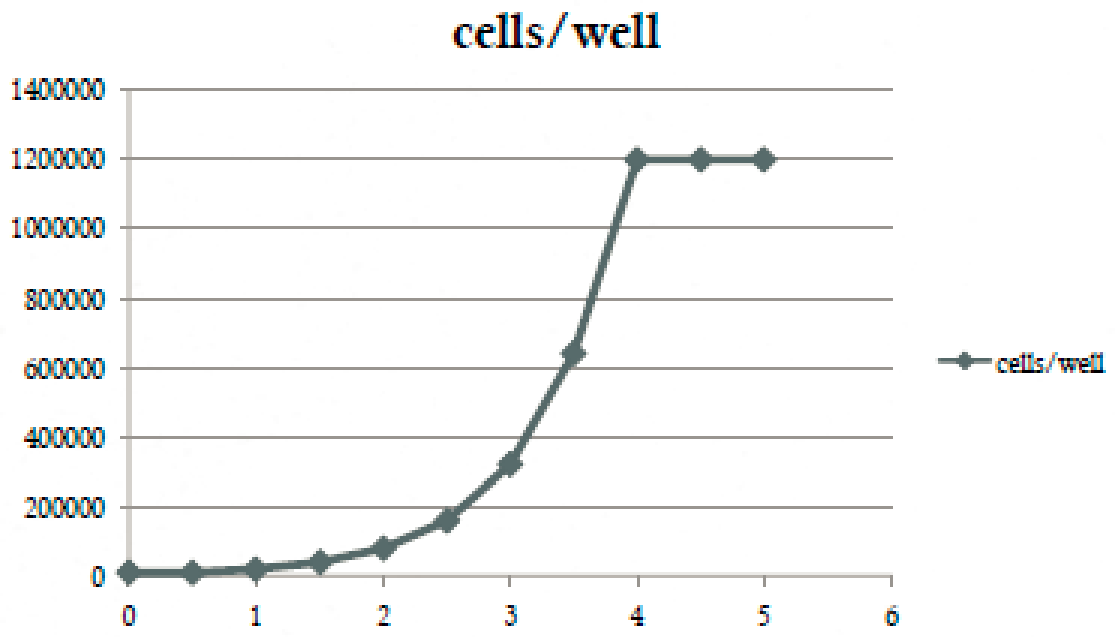
$\text{Log } 10^3 = x$
$10^x = 10^3$
$X = 3$

$\text{Log } (2 \times 10^3) = x$
$10^x = 2 \times 10^3$
$X = 3.3$

Note: consider purchasing semilog graph paper

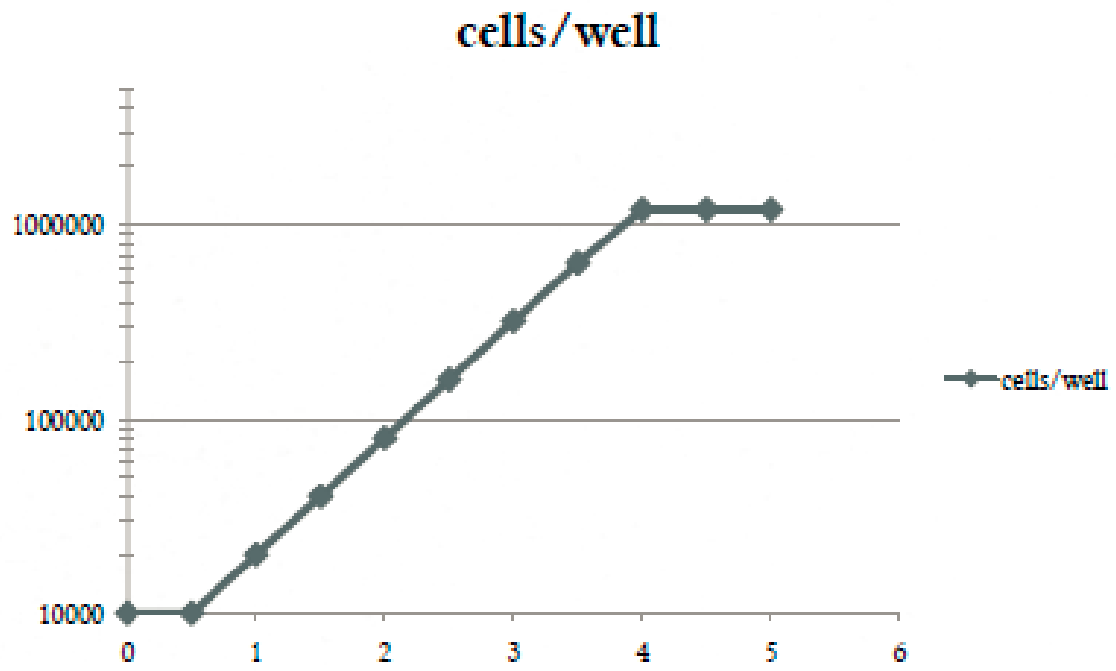
days from subculture	cells/well
0	10000
0.5	10000
1	20000
1.5	40000
2	80000
2.5	160000
3	320000
3.5	640000
4	1200000
4.5	1200000
5	1200000

Most mammalian cells double every ~24 hours in culture. Making their growth exponential over a number of days.



days from subculture	cells/well
0	10000
0.5	10000
1	20000
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Plotting the data on a semi-log scale will straighten the line, allowing for a more accurate identification of doubling time.



Population Doubling Time

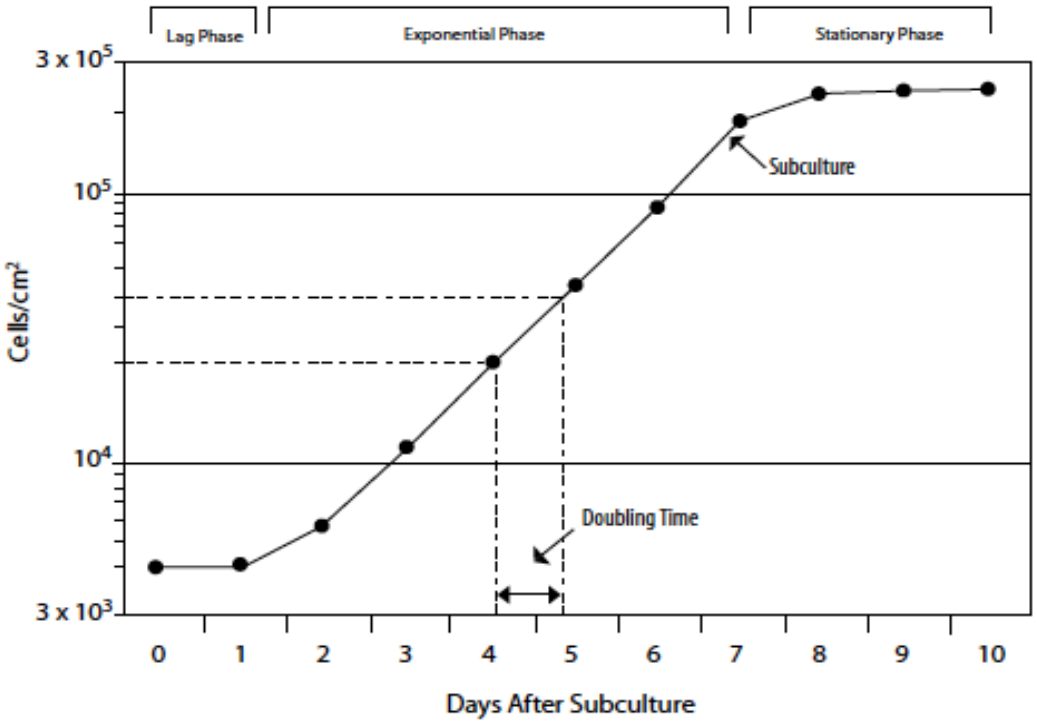


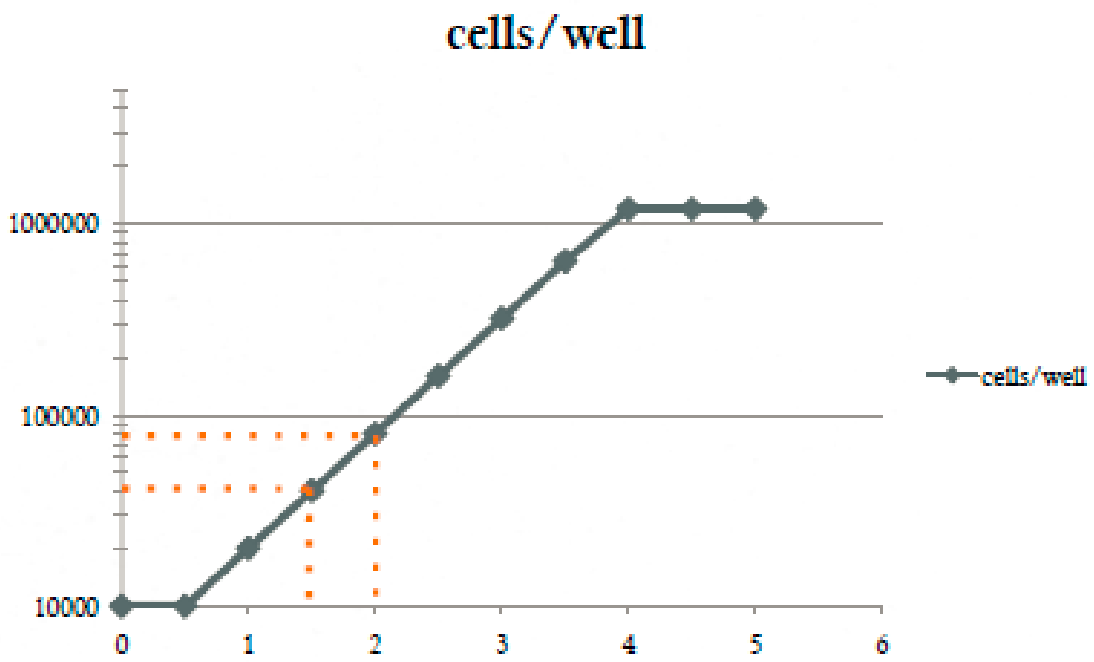
Figure 1. Growth curve for cells grown in culture. Cells should be subcultured while still in the exponential phase.

$$DT = T \ln 2 / \ln(Xe/Xb)$$

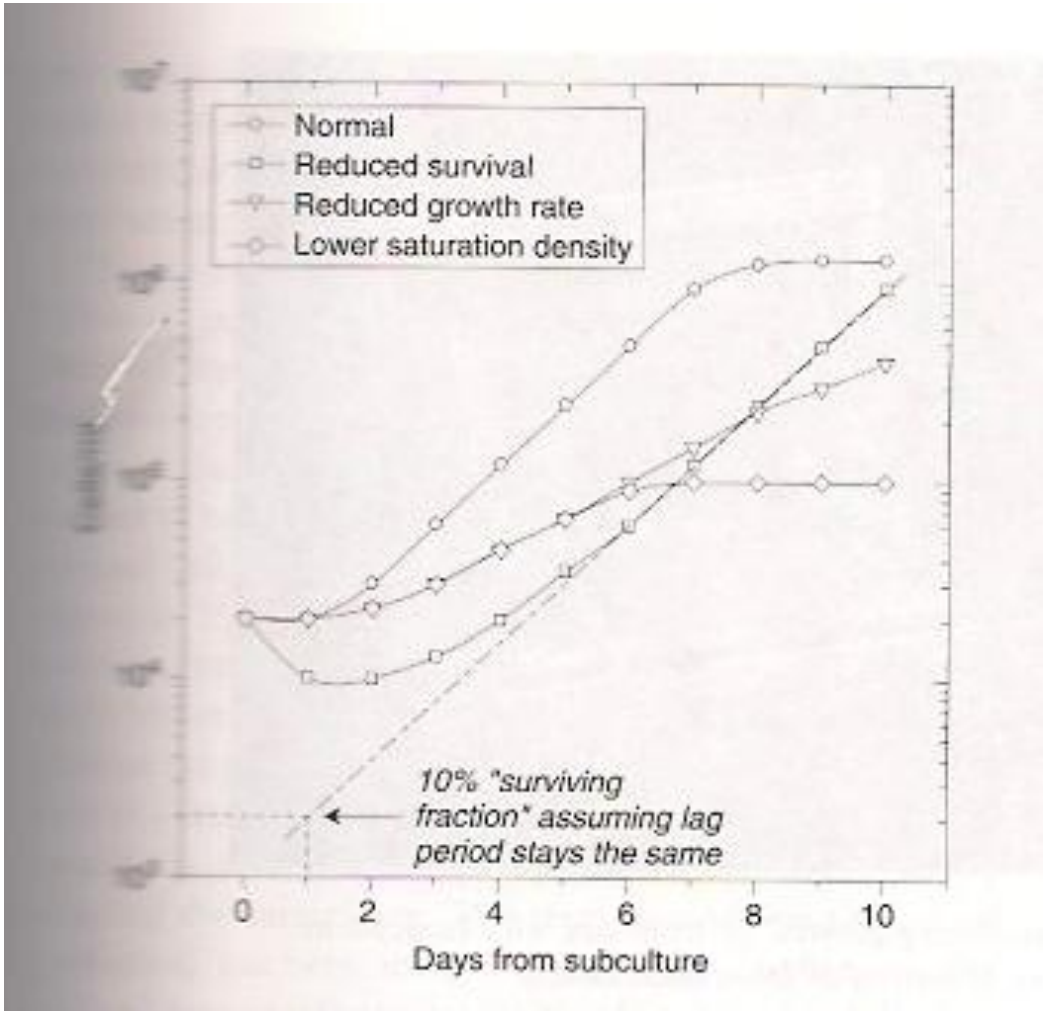
T is the incubation time in any units.
Xb is the cell number at the beginning of the incubation time.
Xe is the cell number at the end of the incubation time.

days from subculture	cells/well
0	10000
0.5	10000
1	20000
1.5	40000
2	80000
2.5	160000
3	320000
3.5	640000
4	1200000
4.5	1200000
5	1200000

What is the population doubling time???



GROWTH CURVE COMPARISONS



This week you will generate a growth curve of your T25 flask of cells.

What other ways can we evaluate cell proliferation?

MEASUREMENTS OF CELL PROLIFERATION

Population Doubling Time: Time for a given cell population to double. Product of cell division, cell death, and non-dividing (senescent) cells.

Growth Curve: Semi-log plot of cell number vs. time from subculture. The growth curve is used to determine population doubling time.

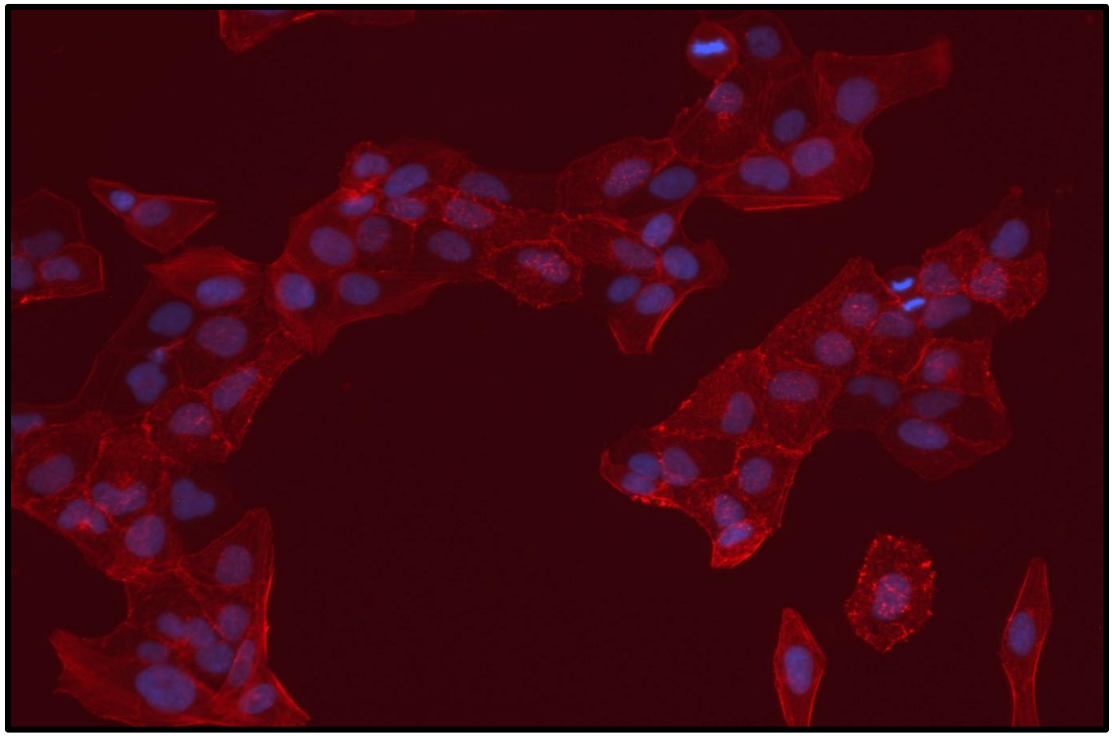
Mitotic Index: % cells in mitosis. Measured by counting mitotic nuclei of cells as a % of the total population.

Viability Index: % of cells that excluding a vital stain such as Trypan Blue.

Plating Efficiency: Number of cells capable of forming discrete colonies following subculture at a low cell density.

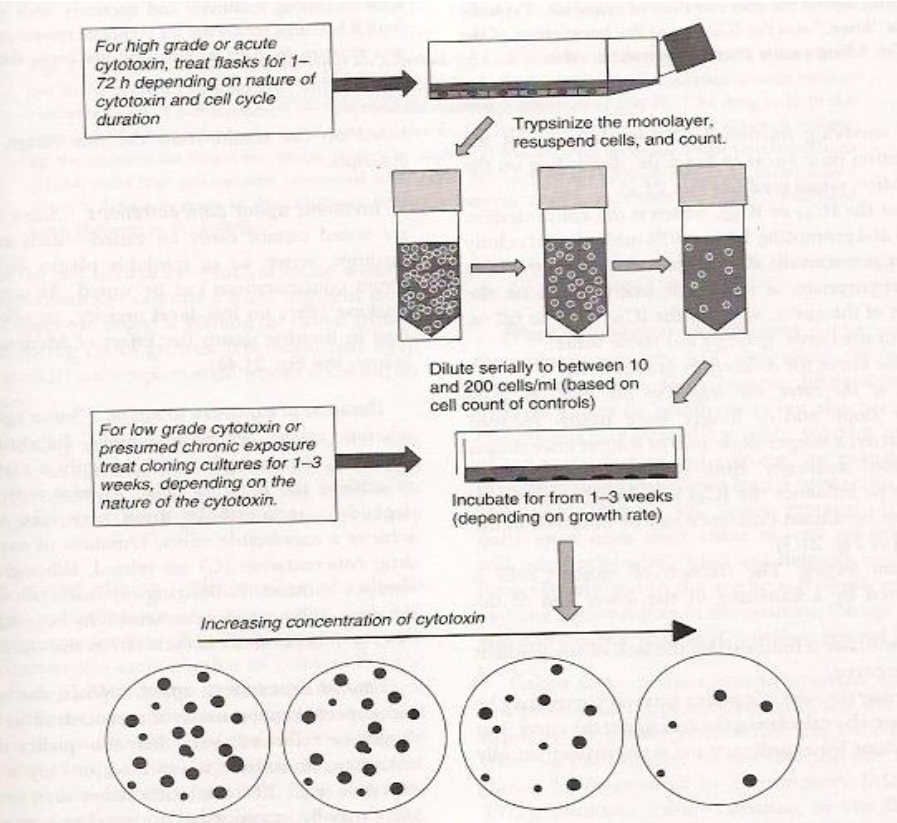
Mitotic Index

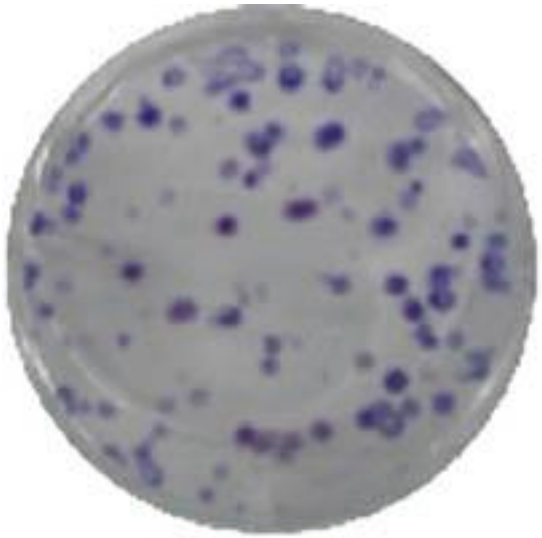
The fraction or percentage of cells in mitosis and is determined by counting mitoses in stained cultures as a proportion of the whole population of cells.



Plating Efficiency

Number of cells capable of forming discrete colonies following subculture at low cell density.



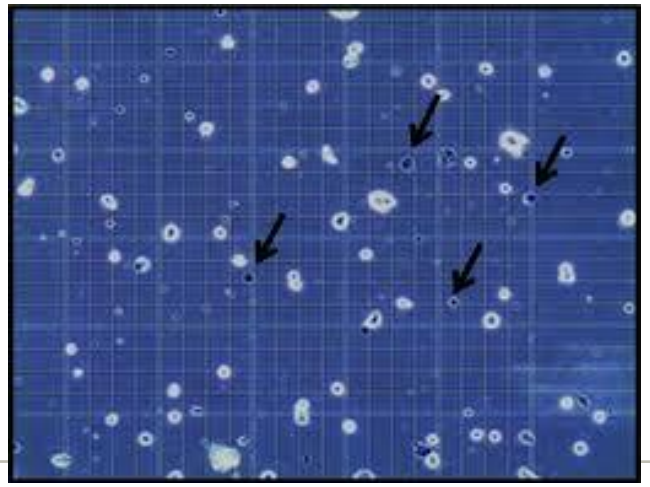


Viability Index
Trypan Blue

• Cells with intact membranes will exclude Trypan Blue dye. Therefore, cells that stain blue are said to have a breakdown of membrane integrity and cell death.

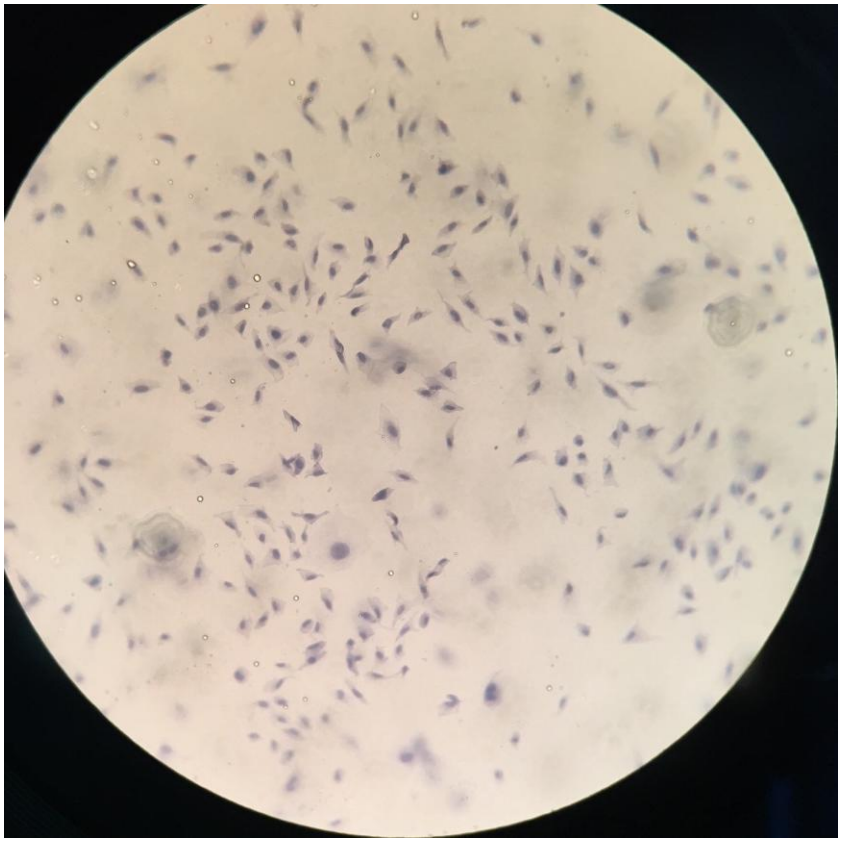
• This assay often UNDER estimates cell death. For example, 90% of cells thawed from liquid nitrogen may exclude Trypan Blue but only 60% prove to be capable of attachment 24hr later.

• May be performed in living cells without killing them. i.e. **vital stain**





IC50 of Ethanol after 15min: Assessed by Trypan Blue

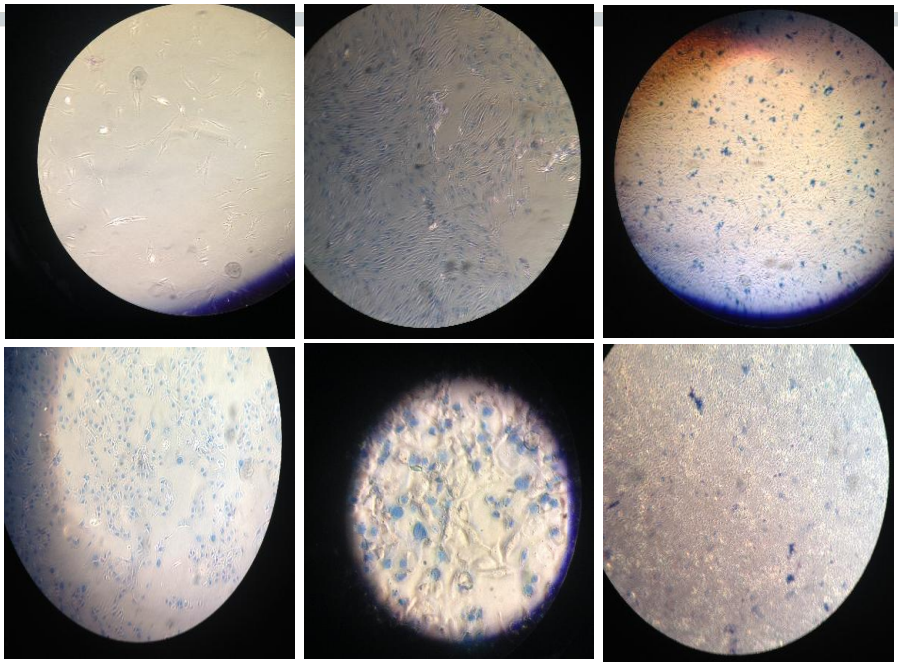


Cell Template: Ethanol percentage and the time when cells appear unhealthy		
%_____ Time_____	%_____ Time_____	%_____ Time_____
%_____ Time_____	%_____ Time_____	%_____ Time_____

Start with a wide range: 0.5%, 5% 50% After the first three, you can try a narrower range.

It is likely that you will see cell death even before adding Trypan Blue.

Trypan Blue Data



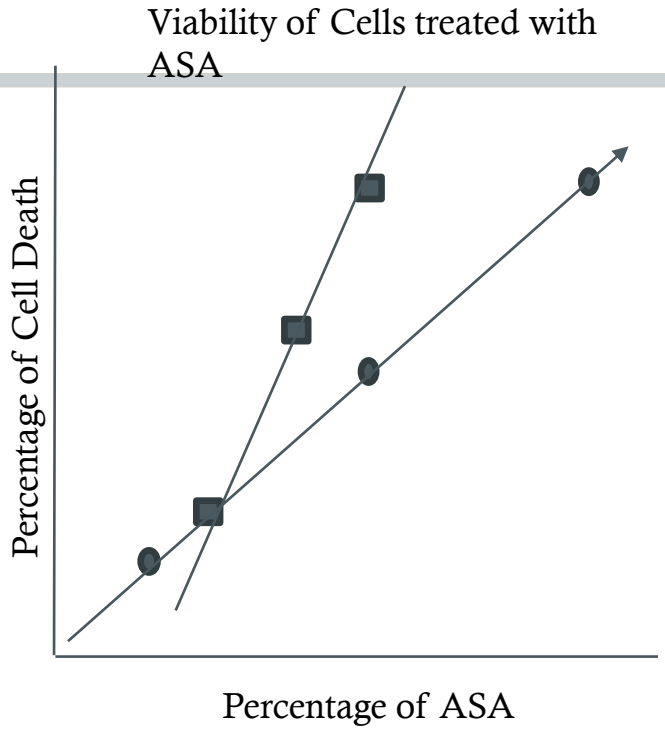
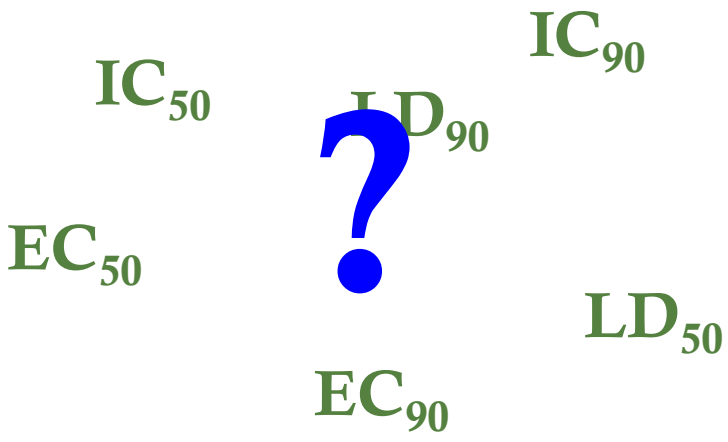


Figure 1. Trypan Blue experiment to find the IC50 of ASA for HFF and U2-OS cells. **A.** HFF cells exposed to 10% ASA. **B.** HFF cells exposed to 100% ASA. **C.** HFF cells exposed to 50% ASA. Approximately 50% of the cells were observed as undergoing cell death. **D.** U2-OS cells exposed to 15% ASA. **E.** U2-OS cells exposed to 35% ASA. Approximately 50% of the cells were undergoing cell death at that concentration. **F.** U2-OS cells exposed to 50% ASA.

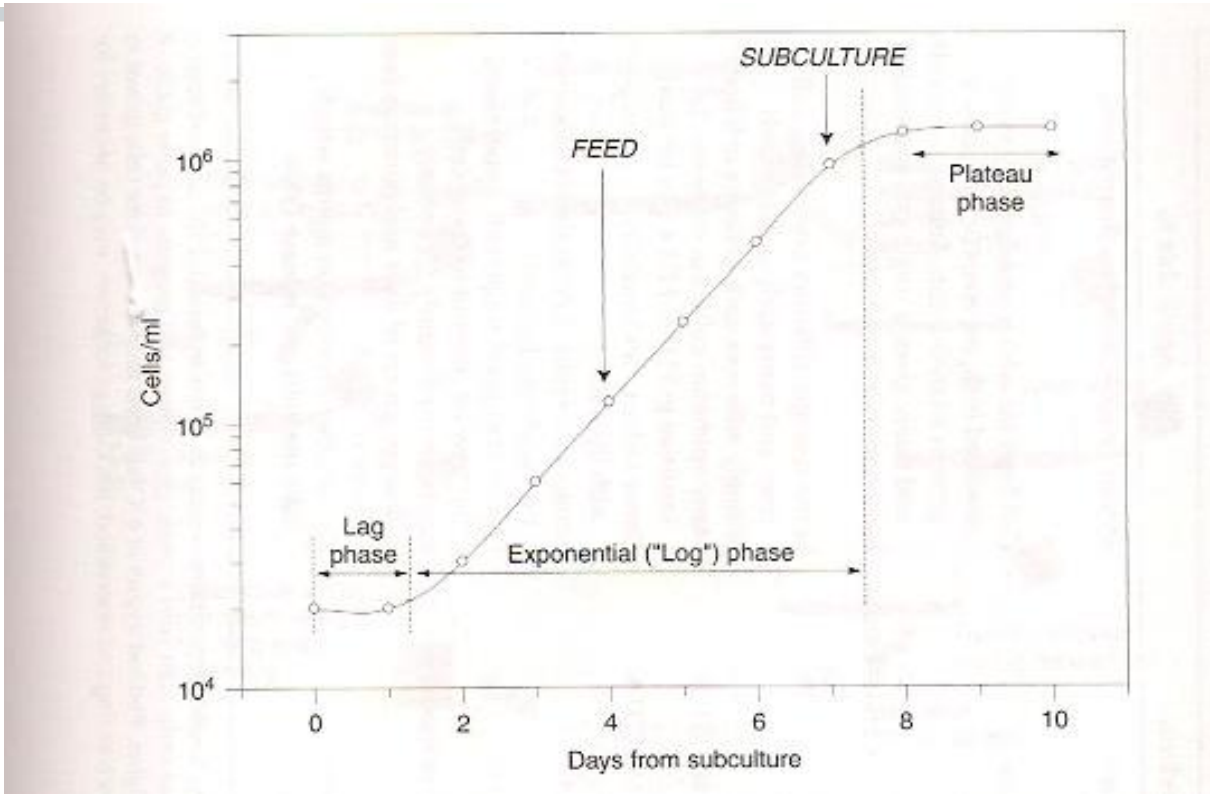


EC_{50}: The half maximal effective concentration. The dose at which the desired response is achieved in 50% of the population. Generally refers to agonist drug in vitro.

IC_{50}: The half maximal inhibitory concentration. The concentration at which 50% of the cell population dies. Generally refers to antagonist drugs in vitro.

LD_{50}: The half maximal lethal dose. The dose at which 50% of the population dies. Generally refers to antagonist drug in vivo.

GROWTH CURVE DATA



1) Estimate percentage of confluence in images taken throughout the week.

2) Convert the percentage of confluence to cell number. Assume there are 2.6 x 10^6 total cells in a 100% confluent flask.

Graph: Total Cell Number (log scale) vs. Days from subculture.

Using Multi-well Plates

This week you split your T25 flask into a 6-well plate.

Next week we will split our T25 flasks into a 96-well plate.

Keep in mind that the growth surfaces have different areas: T25 ($25cm^2$), 6-well ($9cm^2$), 96-well (4.3mm)

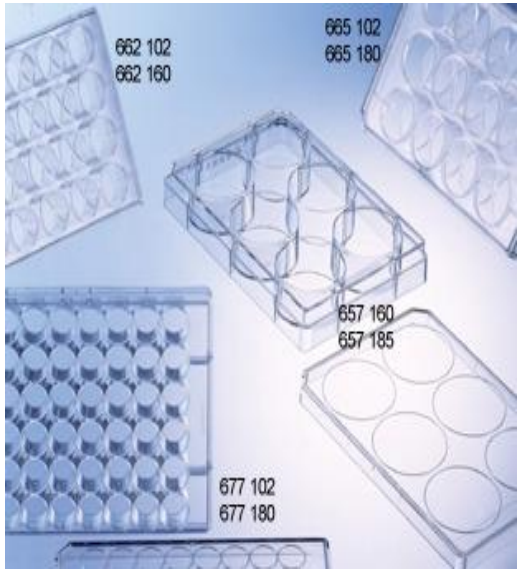
MULTI-WELL PLATES

Purpose: Designed for replicate monolayer cultures in experiments requiring multiple variables and replicates for statistical analysis.

- Available in various sizes
  - 4-well, 6-well, 12-well, 24-well, microtitration (96-well and 144-well)

- Made of plastic
  - single-use, sterile polystyrene multi-well plates
  - provide a simple, reproducible substrate for culture
  - good (but not great) optical quality
    - glass coverslips can be used for better imaging

- Lids designed to allow for gas exchange



SUBSTRATE MATERIALS

Optically, glass medium is the best for cell imaging



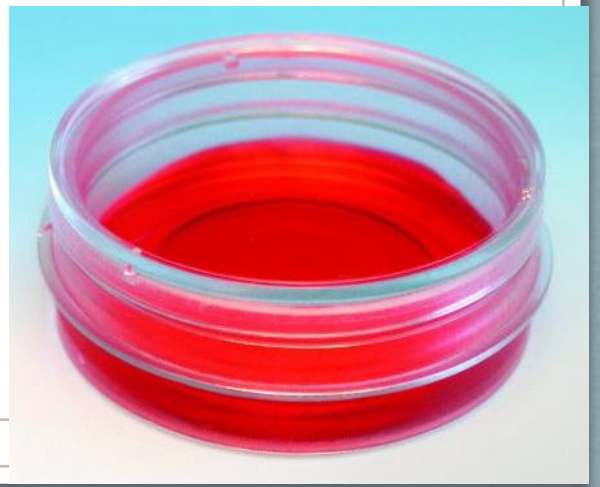
Many cell types will adhere and grow on glass coverslips.

-Place a coverslip in each well before adding cells.

Coverslips can later be removed, flipped over, mounted on slides and imaged.

Glass bottom dishes can also be useful.





MULTI-WELL PLATES

TABLE 7.2. Culture Vessel Characteristics

Culture vessel	Replicates	mL	cm^{2a}	Approximate cell yield (HeLa)
Multiwell plates				
Microtitration	96	0.1	0.3	1×10^5
Microtitration	144	0.1	0.3	1×10^5
4-well plate	4	2	2	5×10^5
6-well plate	6	2	10	2×10^6
12-well plate	12	1	3	7.5×10^5
24-well plate	24	1	2	5×10^5
Petri dishes				
3.5-cm diameter	1	2	8	2×10^6
5-cm diameter	1	4	17.5	4×10^6
6-cm diameter	1	5	21	5×10^6
9-cm diameter	1	10	49	1×10^7
Flasks				
10 cm^2 (T10)	1	2	10	2×10^6
25 cm^2 (T25)	1	5	25	5×10^6
75 cm^2 (T75)	1	20	75	2×10^7
175 cm^2 (T175)	1	50	175	5×10^7
225 cm^2 (T225)	1	75	225	6×10^7
Roller bottle	1	200	850	2.5×10^8
Multisurface propagators				
Nunc Triple flask	1	200	500	1×10^8
Corning HyperFlask	1	560	1720	5×10^8
Nunclon Cell Factory	1	200–8,000	632–25,284	$2–75 \times 10^8$
Corning CellStack	1	150–8,000	636–25,440	$2–75 \times 10^8$
Stirrer bottles				
500 mL (unsparged)	1	50		5×10^7
5000 mL (sparged)	1	4000		4×10^9

[a]These figures are approximate; actual areas will vary by source.