

## Cell Plating Calculator Quick Guide

Axion's Cell Plating Calculator is used to perform dilution calculations for a variety of cell plating conditions.

Included in this guide:

- Quick steps on how to use the tool.
- Definitions for the fields.
- Equations used in our calculations.

### HOW TO USE THE TOOL

1. Enter your cell count (**Cell Stock  $D_{initial}$** ), in cells per mL, and initial cell suspension volume (**Cell Stock  $V_{initial}$** ).

**Note:** An optional box allows you to enter hemocytometer values which will calculate the cell suspension density.

**Step 1**

**Starting conditions:**

Cell Stock  $V_{initial}$   mL ▼

Cell Stock  $D_{initial}$   cells/ml

Hemocytometer (optional)

Total Cells	# of Squares	Dilution
<input type="text"/>	<input type="text"/>	<input type="text"/>

2. Enter the expected **Plating Efficiency**, or the % of cells that will attach. If unknown, This may be set or left at 100%. For multiple plating densities, **Extra V** % provides room for error in volume loss due to pipetting.

**Step 2**

**Target conditions:**

Plating Efficacy  %

Extra V  %

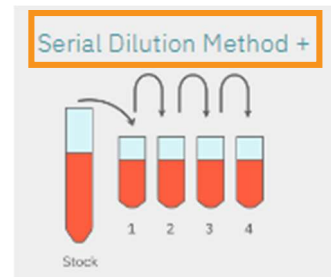
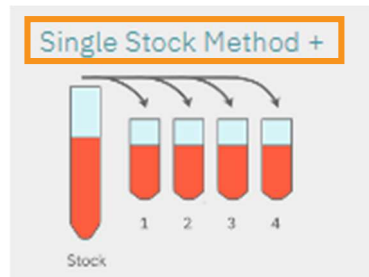
- Enter your target cell plating density in the **Cells per Well** field, as well as the cell plating volume ( $V_{\text{plating}}$ ) and number of wells to be plated. Multiple plating densities may be added or removed with the **plus** and **minus** buttons.

**Note:** Densities must be entered from highest to lowest.

Group	Cells per Well	$V_{\text{plating}}$ ( $\mu\text{L}$ )	# of Wells	
1	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="button" value="+"/>
2	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="button" value="-"/>

- You may choose to prepare aliquots using a **Single Stock Method**, where each aliquot is made from the same starting stock, or a **Serial Dilution Method**, where each aliquot is diluted from the previous aliquot.

**Note:** Click on the aliquot method name to change the method selected.



- Then, click **Calculate Results**. The following outputs are provided

- Total** and **Needed cells** provide the number of cells needed for the experiment.

▼ Results table:

Total cells
Needed cells

- **Cell Stock  $V_{\text{final}}$**  provides the final volume to dilute (green alert with additional volume of media to add) or concentrate (red warning that you do not have enough cells) your sample to achieve the highest cell density chosen.
- The dilution table on the right shows how to prepare each aliquot, including how much stock or aliquot is required, how much needs to be diluted, and the volume of the final aliquot.

▼ Dilution table:

Single Stock			
*Group	$V_{\text{cells}}$ ( $\mu\text{L}$ )	$V_{\text{media}}$ ( $\mu\text{L}$ )	$V_{\text{final}}$ ( $\mu\text{L}$ )
1	17.6	0	17.6
2	8.8	8.8	17.6

*\* Prepare each aliquot from Cell Stock*



## DEFINITIONS

### Starting Conditions

Cell $V_{initial}$	Initial cell suspension volume.
Cell $D_{initial}$	Initial cell density. Can be entered by the user or calculated from hemocytometer.
<u>Hemocytometer:</u>	
Total Cells	Total viable cells counted on all squares.
#Squares	Number of large (4x4) squares counted.
Dilution	Cell:Trypan Blue dilution factor.

### Target Conditions

Plating Efficiency	Percentage of cells attached/cells added.
Extra V	Percentage of overfill each aliquot will get.
Single Stock Method	Each aliquot is made from the same starting stock.
Serial Dilution Method	Each aliquot is diluted from the previous aliquot.
Cells per well	Target cells per well.
$V_{plating}$	Target plating volume ( $\mu$ l).
# Wells	How many wells to plate at this density.

### Results

Total cells	Total starting cells.
Needed cells	Total cells needed to plate all wells at specified densities. Factors in extra V% and plating efficiency.
Cell Stock $V_{final}$	Final cell stock volume to dilute or concentrate to. <i>Tip: If Total Cells &gt;&gt; Needed Cells, reduce <math>V_{initial}</math> to conserve media.</i>
Cell Stock $D_{final}$	Final cell stock density.

## EQUATIONS USED IN OUR CALCULATIONS

### Results calculations:

(*n* = group number)

$$\text{Total cells} = \text{Cell } D_{\text{initial}} * \text{Cell } V_{\text{initial}}$$

$$\text{Needed cells} = \left( \sum_1^n \left( \frac{\text{cells}}{\text{well}} \right)_n * (\# \text{ of wells})_n \right) * (\text{Extra } V/100) / (\text{Plating Efficiency}/100)$$

$$\text{Cell Stock } V_{\text{final}} = (\text{Total cells}) * \left( \frac{\text{Plating Efficiency}}{100} \right) * \left( \frac{V_{\text{plating}}}{\frac{1000}{\left( \frac{\text{cells}}{\text{well}} \right)}} \right)$$

$$\text{Cell Stock } D_{\text{final}} = \left( \frac{\text{cells}}{\text{well}} \right)_1 / V_{\text{plating}_1}$$

### Dilution calculations:

Single Stock

(*n* = group number)

$$V_{\text{cells}_n} = \frac{\frac{\text{cells}}{\text{well}}_n}{V_{\text{plating}_n}} * \frac{V_{\text{final}_n}}{\text{Cell Stock } D_{\text{final}}}$$

$$V_{\text{final}_n} = V_{\text{plating}_n} * \# \text{ of wells}_n * \left( 1 + \left( \frac{\text{Extra } V}{100} \right) \right)$$

$$V_{\text{media}_n} = V_{\text{final}_n} - V_{\text{cells}_n}$$

Serial Dilution

(*n* = group number, *n*=0 is the cell stock)

$$V_{\text{cells}_n} = \frac{\left( \frac{\text{cells}}{\text{well}} \right)_n * V_{\text{final}_n}}{\left( \left( \frac{\text{cells}}{\text{well}} \right)_{n-1} / V_{\text{plating}_{n-1}} \right)}$$

$$V_{\text{final}_n} = V_{\text{plating}_n} * \# \text{ of wells} * \left( 1 + \left( \frac{\text{Extra } V}{100} \right) \right) + V_{\text{final}_{n+1}}$$

$$V_{\text{media}_n} = V_{\text{final}_n} - V_{\text{cells}_n}$$

