AXION BIOSYSTEMS

Drug Dosing Calculator

Version 202608

Contents

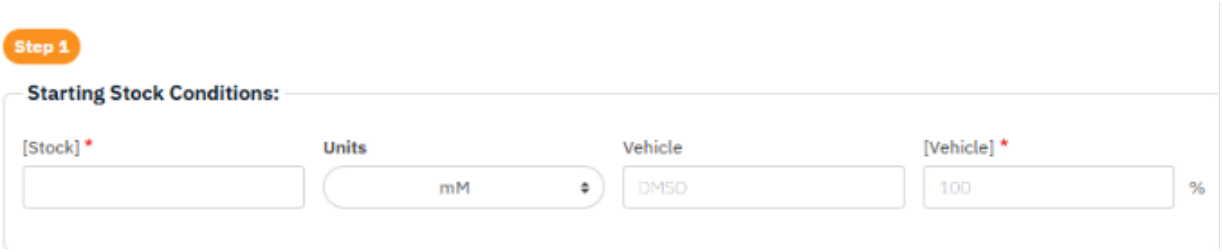
Chapter 1. Overview

1.1. Drug Dosing Calculator Overview

Axion's Drug Dosing Calculator calculates the concentrations and volumes needed to prepare dosing solutions for plate-based experiments.

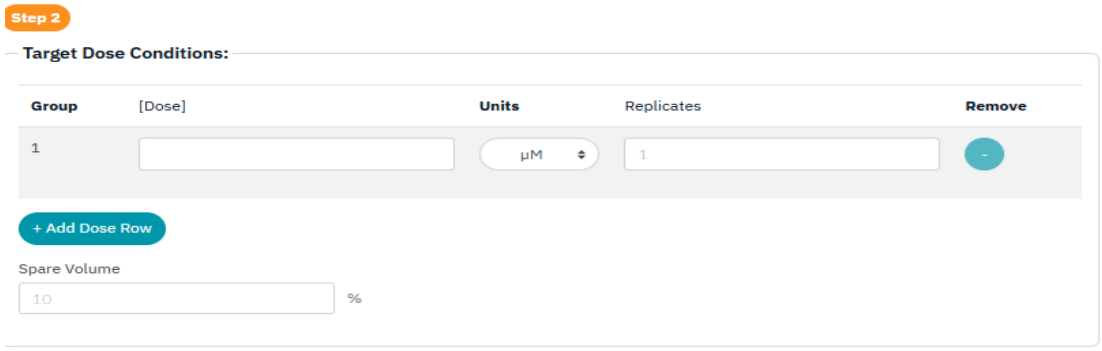
1.2. Stock Solution Conditions

1. Enter your concentrated stock solution **(Stock)** and the vehicle concentration in the stock solution **(Vehicle).**
    1.1 Ensure proper units are selected using the dropdown option.
    1.2 *For example, a compound prepared in all DMSO would typically need to be entered as 100% DMSO.*



1.3. Target Dose Conditions

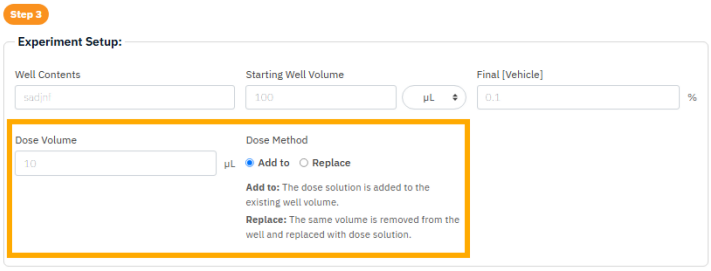
1. Enter your target cell plating density in the **Cells per Well** field, as well as the cell plating volume **($V_{plating}$)** and number of wells to be plated.
2. Multiple plating densities may be added or removed with the **plus** and **minus** buttons.
    2.1 ***Note:*** *Order the list of dosage concentrations with proper units from highest to lowest.*
    2.2 *Additionally, Spare Volume provides room to account for extra solution in each aliquot due to pipetting loss or dead volume.*



1.4. Well Conditions

1. Enter your well-starting solution volume **(Starting Well Volume)** and the % of desired final vehicle concentration into **Final [Vehicle]**.
   1.1. **Dose Volume** represents the volume of drug solution added to the well.

2. To add to the initial volume or replace an equivalent amount of volume with dose solution, click on ***Add To*** or ***Replace*** respectively.



1.5. Solution Results

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

1. **Product** identifies the solution being prepared.
2. **[Conc]** provides the target concentration of the product.
3. Solution identifies the source solution used to prepare the product.
4. $\mathbf{V_{solution}}$ provides the required volume of the source solution.
   4.1. It is rounded to the nearest 0.1 µL and is not allowed to be less than 10 µL. $V_{diluent}$ is adjusted accordingly.
5. **Diluent** identifies the diluent used to prepare the product.
6. $\mathbf{V_{diluent}}$ provides the required volume of diluent.

For each solution (Product) you need to make, add $\mathbf{V_{diluent}}$ to $\mathbf{V_{solution}}$ slowly, mixing as needed.

> ***Note:*** *Adding* $\boldsymbol{V_{solution}}$ *to* $\boldsymbol{V_{diluent}}$ *may cause solubility issues. Always dilute high to low.*

> ***Note:*** *The starting stock or vehicle concentration is considered too low if the desired final vehicle concentration cannot be achieved.*

▼ **Working Solution Preparation**

Product	[Conc]	$V_{solution}$	Solution	$V_{diluent}$	Diluent
Work 1	55 µM	10 µL	Int 1	80.9 µL	Dil 1

Definitions

2.1. Starting Conditions

Stock Solution Conditions	
[Stock]	Concentration of the stock solution
Vehicle	Vehicle control
[Vehicle]	Concentration of the vehicle in the stock solution

2.2. Target Conditions

Target Dose Conditions	
[Dose]	Final concentration in the well
Replicates	Number of wells to receive the dose
Spare Volume	Percentage of overfill each aliquot will get to avoid running short.

2.3. Well Conditions

Experiment Setup	
Well Contents	Solution or medium present in the well before dosing.
Starting Well Volume	Volume of solution or medium present in each well before dosing.
[Vehicle]	Final concentration of the vehicle in percent
Dose Volume	Volume of drug solution added to the well
Dose Method	Method of dose addition

2.4. Results

Results	
Product	The final solution being prepared
[Conc]	Final concentration of the solution being prepared
$V_{solution}$	Required volume of the source solution
Solution	Source solution used to prepare the product
$V_{diluent}$	Required volume of diluent
Diluent	Diluent used to prepare the product

Chapter 3. Equations

3.1. Solution Design

Solution Design Calculations:
(n = number of drug solutions or length of the array)

$$Number\ of\ Intermediates = Round\frac{(Log(Concentration_{stock} - Log(Concentration_{work_1})}{2}$$

$$Concentration_{working_1} = \frac{(Concentration_{dose_n} * V_{well\,final})}{V_{add}}$$

For Add: $V_{well\,final} = V_{well} + V_{add}$

For Replace: $V_{well\,final} = V_{well}$

If $(\frac{Log(Concentration_{stock} - Log(Concentration_{work_1}))}{2} - (x-1) \geq 1\ then\ C_{drug_n} = \frac{C_{drug_{n-1}}}{100}$

Else if $\frac{Log(Concentration_{stock} - Log(Concentration_{work_1}))}{2} - (x-1)\ \geq 0.5\ and\ \leq then\ C_{drug_n} = \frac{C_{drug_{n-1}}}{10}$ 0

Volume Calculations

For working solutions: $(V_{add} * Rep + Vs_{n+1}) * (1 + \frac{V_{extra}}{100})$

For intermediates:

If $Vf_n \leq (1000\ ul)\ then\ Vf_n = 1000$

Else $Vf_n = (V_{add} * Rep + Vs_{n+1}) * (1 + (\frac{V_{extra}}{100}))$

$$Vs_n = \frac{(C_{drug_n} * Vf_n)}{(C_{drug_{n-1}}}$$

$$V_{d_n} = V_{f_n} - V_{s_n}$$

Vehicle & Diluent Preparation Calculations:

$$Concentration_{vehicle_{working}} = \frac{Concentration_{vehicle_{final}} * V_{well_{final}}}{V_{add}}$$

$$C_{diluent_1} = C_{vehicle_{working}} - C_{vehicle_n} \; where \; n = the \; first \; solution \; where \; C_{vehicle_n} \leq C_{vehicle_{working}}$$

$$Vf_{diluent_1} = Vd_n * \left(1 + \left(\frac{V_{extra}}{100}\right)\right) where \; n = the \; first \; solution \; where \; C_{vehicle_n} \leq C_{vehicle_{working}}$$

$$Vs_{diluent_1} = \frac{C_{diluent_1} * Vf_{diluent_1}}{100}$$

$$Vd_{diluent_2} = Vf_{diluent_2} - Vs_{diluent_2}$$