

How to use the Cell & Reagent Calculator for the 96-well Shuttle®:

<http://www.amaxa.com/cellreagentcalculator.html>

1) Fill in the top 2 sections based on your experimental set up and stock solutions.

a) General

GENERAL	Amount	Unit	Default
number of transfections	<input type="text"/>	#	96
cells / transfection	<input type="text"/>	#	
void volume comp. 20µl + X %	<input type="text"/>	%	20

- Fill in the number of transfections you wish to perform, i.e. how many wells you will use. It is generally recommended to do increments of 16 wells.
- Fill in the number of cells you will load per transfection (per well)
- The void volume determines the amount of overage to allow for pipetting. The suggested default is 20% which is generally sufficient when using a multichannel pipette and one row of a v-bottom plate as trough for preparing the solution to transfer to the 96-well Nucleocuvette™ plate.

b) Substrates

SUBSTRATES			
pmaxGFP (0.2µg/µl) / reaction	<input type="text"/>	µg	
pmaxGFP concentration (stock)	0.2	µg/µl	
DNA / transfection	<input type="text"/>	µg	
DNA concentration (stock)	<input type="text"/>	µg/µl	
siRNA / transfection in µg	<input type="text"/>	µg	
siRNA concentration (stock)	<input type="text"/>	µg/µl	
siRNA / transfection in µM	<input type="text"/>	µM	
siRNA concentration (stock)	<input type="text"/>	µM	

- Fill in the amount per reaction and stock concentration for the substrate you wish to use. The concentration of the pMAXGFP control is already entered. For DNA transfections, enter the amount you wish to use per reaction in µg and the concentration of the stock in µg/µl. For siRNA, you may either enter the µg per reaction and stock as µg/µl or the final concentration in a reaction as µM and the stock solution as µM.

2) Press calculate to display the results in Substrate Volumes, Solutions and Cells.

SUBSTRATE VOLUMES			
pmaxGFP (/well)	<input type="text"/>	µl	
pmaxGFP (total)	<input type="text"/>	µl	
DNA (/well)	<input type="text"/>	µl	
DNA (total)	<input type="text"/>	µl	
siRNA (µg-based, /well)	<input type="text"/>	µl	
siRNA (µg-based, total)	<input type="text"/>	µl	
siRNA (µM-based, /well)	<input type="text"/>	µl	
siRNA (µM-based, total)	<input type="text"/>	µl	
SOLUTIONS			
Nucleofector Solution	<input type="text"/>	µl	
Supplement	<input type="text"/>	µl	
total volume	<input type="text"/>	µl	
CELLS			
cell number in total volume	<input type="text"/>	#	

- The volume of substrate per well is calculate as is the total volume including the 20% overage allowing you to prepare individual samples or a master mix should all wells be getting the same substrate.
- The solution values will tell the total amount of supplement and Nucleofector solution to mix for all wells and allow for the overage.
- The cells value will be total needed cells to resuspend in the total Nucleofector solution plus supplement.

3) Rinsing Medium and Cell Culture Post Transfection

These sections can be used to quickly calculate the amount of media to have prewarmed and preplated for post-nucleofection. Enter the volume to be added per well post-nucleofection in the rinsing section and press calculate to get the total prewarmed media needed for adding directly to the nucleocuvette. Enter the Transfer Volume and total plating volume in the Cell culture section to determine the amount to plate prior to beginning nucleofection.

RINSING MEDIUM POST TRANSFECTION			
medium / well post transfection	<input type="text"/>	µl	80
min. vol. of prewarmed medium	<input type="text"/>	ml	
CELL CULTURE POST TRANSFECTION			
transfer volume to cell culture	<input type="text"/>	µl	25/50
total cell culture volume	<input type="text"/>	µl	
preplated medium volume / well	<input type="text"/>	µl	

4) Example

In the example below, we will be preparing for an experiment with Jurkat cells with 32 samples using siRNA at a final concentration per reaction of 0.5 μM and following the protocol recommendations of 200,000 cells per reaction, 80 μl media added post-nucleofection, and 50 μl transferred.

GENERAL	Amount	Unit	Default
number of transfections	32	#	96
cells / transfection	200000	#	
void volume comp. 20 μl + X %	20	%	20
SUBSTRATES			
pmaxGFP (0.2 $\mu\text{g}/\mu\text{l}$) / reaction		μg	
pmaxGFP concentration (stock)	0.2	$\mu\text{g}/\mu\text{l}$	
DNA / transfection		μg	
DNA concentration (stock)		$\mu\text{g}/\mu\text{l}$	
siRNA / transfection in μg		μg	
siRNA concentration (stock)		$\mu\text{g}/\mu\text{l}$	
siRNA / transfection in μM	0.5	μM	
siRNA concentration (stock)	20	μM	
SUBSTRATE VOLUMES			
pmaxGFP (/well)		μl	
pmaxGFP (total)		μl	
DNA (/well)		μl	
DNA (total)		μl	
siRNA (μg -based, /well)		μl	
siRNA (μg -based, total)		μl	
siRNA (μM -based, /well)	0.6	μl	
siRNA (μM -based, total)	19.2	μl	
SOLUTIONS			
Nucleofector Solution	665.9	μl	
Supplement	147.5	μl	
total volume	768.0	μl	
CELLS			
cell number in total volume	7.68e+6	#	
RINSING MEDIUM POST TRANSFECTION			
medium / well post transfection	80	μl	80
min. vol. of prewarmed medium	2.56	ml	
CELL CULTURE POST TRANSFECTION			
transfer volume to cell culture	50	μl	25/50
total cell culture volume	200	μl	
preplated medium volume / well	150	μl	