

# How to Use the Cell & Reagent Calculator for the Nucleofector™ 96-well Shuttle™ Technical Information

[www.lonza.com/cell-reagent-calculator](http://www.lonza.com/cell-reagent-calculator)

## 1) Fill in the Top 2 Sections Based on Your Experimental Set Up and Stock Solutions

### a) General

- 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 repository for preparing the solution to transfer to the 96-well Nucleocuvette™ Plate.

| 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      |

### b) Substrates

- Fill in the amount per reaction and stock concentration for the substrate you wish to use. The concentration of pmaxGFP™ Vector 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.

| SUBSTRATES                     |                                  |       |
|--------------------------------|----------------------------------|-------|
| pmaxGFP™ / reaction            | <input type="text"/>             | µg    |
| pmaxGFP™ concentration (stock) | <input type="text" value="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    |

## 2) Press Calculate to Display the Results in Substrate Volumes, Solutions and Cells

- The volume of substrate per well is calculated including 20% overage. This allows you to either prepare individual samples or a master mix in case all samples will get 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 represents the total number of cells to resuspend in the total supplemented Nucleofector™ Solution.

| SUBSTRATE VOLUMES           |                      |    |
|-----------------------------|----------------------|----|
| pmaxGFP™ (per well)         | <input type="text"/> | µl |
| pmaxGFP™ (total)            | <input type="text"/> | µl |
| DNA (per well)              | <input type="text"/> | µl |
| DNA (total)                 | <input type="text"/> | µl |
| siRNA (µg-based, per well)  | <input type="text"/> | µl |
| siRNA (µg-based, total)     | <input type="text"/> | µl |
| siRNA (µM-based, per 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"/> | #  |

## 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™ Plate. 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 per 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 per 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  $\mu\text{M}$  and following the protocol recommendations of 200,000 cells per reaction, 80  $\mu\text{l}$  media added post Nucleofection™, and 50  $\mu\text{l}$  transferred.

| GENERAL                                  | Amount                              | Unit | Default |
|--|-------------------------------------|------|---------|
| number of transfections                  | <input type="text" value="32"/>     | #    | 96      |
| cells / transfection                     | <input type="text" value="200000"/> | #    |         |
| void volume comp. 20 $\mu\text{l}$ + X % | <input type="text" value="20"/>     | %    | 20      |

| SUBSTRATES                            |                                  |                           |  |
|---------------------------------------|----------------------------------|---------------------------|--|
| pmaxGFP™ / reaction                   | <input type="text"/>             | $\mu\text{g}$             |  |
| pmaxGFP™ concentration (stock)        | <input type="text" value="0.2"/> | $\mu\text{g}/\mu\text{l}$ |  |
| DNA / transfection                    | <input type="text"/>             | $\mu\text{g}$             |  |
| DNA concentration (stock)             | <input type="text"/>             | $\mu\text{g}/\mu\text{l}$ |  |
| siRNA / transfection in $\mu\text{g}$ | <input type="text"/>             | $\mu\text{g}$             |  |
| siRNA concentration (stock)           | <input type="text"/>             | $\mu\text{g}/\mu\text{l}$ |  |
| siRNA / transfection in $\mu\text{M}$ | <input type="text" value="0.5"/> | $\mu\text{M}$             |  |
| siRNA concentration (stock)           | <input type="text" value="20"/>  | $\mu\text{M}$             |  |

| SUBSTRATE VOLUMES                       |                                   |               |  |
|---|-----------------------------------|---------------|--|
| pmaxGFP™ (per well)                     | <input type="text"/>              | $\mu\text{l}$ |  |
| pmaxGFP™ (total)                        | <input type="text"/>              | $\mu\text{l}$ |  |
| DNA (per well)                          | <input type="text"/>              | $\mu\text{l}$ |  |
| DNA (total)                             | <input type="text"/>              | $\mu\text{l}$ |  |
| siRNA ( $\mu\text{g}$ -based, per well) | <input type="text"/>              | $\mu\text{l}$ |  |
| siRNA ( $\mu\text{g}$ -based, total)    | <input type="text"/>              | $\mu\text{l}$ |  |
| siRNA ( $\mu\text{M}$ -based, per well) | <input type="text" value="0.6"/>  | $\mu\text{l}$ |  |
| siRNA ( $\mu\text{M}$ -based, total)    | <input type="text" value="19.2"/> | $\mu\text{l}$ |  |

| SOLUTIONS              |                                    |               |  |
|------------------------|------------------------------------|---------------|--|
| Nucleofector™ Solution | <input type="text" value="628.2"/> | $\mu\text{l}$ |  |
| Supplement             | <input type="text" value="139.8"/> | $\mu\text{l}$ |  |
| total volume           | <input type="text" value="768.0"/> | $\mu\text{l}$ |  |

| CELLS                       |                                    |   |  |
|-----------------------------|------------------------------------|---|--|
| cell number in total volume | <input type="text" value="76e+6"/> | # |  |

| RINSING MEDIUM POST TRANSFECTION  |                                   |               |    |
|-----------------------------------|-----------------------------------|---------------|----|
| medium per well post transfection | <input type="text" value="80"/>   | $\mu\text{l}$ | 80 |
| min. vol. of prewarmed medium     | <input type="text" value="2.56"/> | ml            |    |

| CELL CULTURE POST TRANSFECTION   |                                  |               |       |
|----------------------------------|----------------------------------|---------------|-------|
| transfer volume to cell culture  | <input type="text" value="50"/>  | $\mu\text{l}$ | 25/50 |
| total cell culture volume        | <input type="text" value="200"/> | $\mu\text{l}$ |       |
| preplated medium volume per well | <input type="text" value="150"/> | $\mu\text{l}$ |       |

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