Thank you for choosing to use our product. Please note that suboptimal concentrations of DNA and/or reagent may result in unexpectedly high toxicity to your cells, as is the case with many products of this nature. **In order to provide the highest possible transfection efficiency, it is imperative to optimize our product to work best with your specific cells. PLEASE READ SECTION 1.2 AND SEE TABLES 2a AND 2b PRIOR TO PERFORMING ANY TRANSFECTIONS ON YOUR CELLS.** Once you have determined the optimal concentrations of reagent, DNA, and cell number, you may proceed to the next step of the protocol. Please note, these concentrations are guidelines and routinely work well for many cell types. It is possible that certain cells may require one or more additional groups (Continuum & DNA) in order to ensure proper optimization. However, this is only recommended if our optimization suggestions do not produce the expected results.

Important Guidelines for Transfection

1. Prepare high-quality plasmid DNA at 0.5-5 µg/µl in deionized water or TE buffer. A GFP (green fluorescent protein) plasmid can be used to determine transfection efficiency.
2. Use regular high glucose DMEM without serum to make Continuum™ and nucleic acid mix. Do not use $NaCl_2$ solution or PBS.
3. Maintain the same seeding conditions between experiments. Use low passage cells; make sure that cells are healthy and greater than 90% viable before transfection.
4. It is important to have the cells in proliferation state and 70-90% confluence at the time of DNA transfection.
5. Increasing the number of cells plated per well or decreasing the Continuum™ amount will minimize the effect of transfection on cell growth and viability. With careful optimization, this can be achieved while keeping the highest transfection efficiency.
6. Do not use antibiotics in the culture medium during the first 24 hours of transfection.

Protocol

DNA Transfection

1.1 Cell Seeding: For optimal DNA transfection conditions, we recommend using cells which are 70% to 90% confluent at the time of transfection. Typically, for experiments in 6-well plates, 150,000-250,000 adherent cells are seeded per well in 2ml of cell growth medium without antibiotics 24 hours prior to transfection. For the different culture formats, refer to Table 1.

Table 1. Recommended number of cells to seed the day before transfection in culture medium without antibiotics:

Culture Vessel	Number of Adherent cells to seed (Suspension Cells)	Surface Area per well (cm^2)	Volume of medium per well to seed the cells (ml)
96-well	7,500-10,000 ($1x10^5$)	0.3	0.1
24-well	50,000-80,000 ($5x10^5$)	1.9	0.5
12-well	80,000-150,000 ($1x10^6$)	3.8	1
6 well / 35mm	150,000-250,000 ($2x10^6$)	9.4	2
60mm / flask 25 cm^2	250,000-800,000 ($4.5x10^6$)	25-28	5
100mm / flask 75 cm^2	$1x10^6$-$2x10^6$ ($1.4x10^7$)	75-78.5	10
150 mm / flask 175 cm^2	$2x10^6$-$5x10^6$ ($3.5x10^7$)	153-175	25

1.2 DNA Transfection: If this is the first time that you are using Continuum™ on a specific type of cells, first, transfect the cells according to Table 2a for optimization **(The optimization procedures are crucial for successful transfection. Different cell types can have different levels of sensitivity to Continuum™; therefore the amount of Continuum™ needed for maximum transfection can drastically vary on different cell types.)**.

Table 2a. Transfection optimization guidelines according to the cell culture vessel per well.

Component	96-well	24-well	6-well
DMEM w/o Serum (µl)	250	250	1200
DNA (µg)	1.25	1.25	6
Diluted DNA (µl)	4 x 50 µl	4 x 50 µl	4 x 250 µl
Continuum™ (µl)	*0.2, *0.5, *0.7, 1.0	*0.2, *0.5, *0.7, 1.0	1.0, 2.5, 3.8, 5.0
Incubate for 15 min at room temperature			
DNA-reagent complex/well (µl)	10	50	250
Incubate cells for 24 hours or more at 37˚C before analysis.			

**Dilute Continuum™ 1:5 with H_2O prior to application (4 µl reagent + 16 µl H_2O), and then use 5 times of the volumes in the table for accurate pipetting.*

The following table shows the amount of DNA and Continuum™ per well used in each of the above transfection reactions. For additional information on scaling your transfection reaction, see Table 3.

Table 2b. Amount of DNA and Continuum™ per well.

Amount	96-well	24-well	6-well
DNA/well (ng)	50	250	1250
Continuum™ (µl)	0.04-0.2	0.2-1.0	1.0-5.0

As an example, the following steps are provided per well of a 6-well plate optimization. For other culture formats, please refer to Table 2b and Table 3.

1. Transfer 6 µg DNA into 1200 µl regular high glucose DMEM without serum. Mix by vortexing. Aliquot 4 x 250 µl of the above DNA solution into 4 x 1.5 ml Eppendorf tubes.
2. Briefly vortex Continuum™, and add 1.0, 2.5, 3.8, and 5.0 µl into the above diluted DNA respectively. Immediately vortex for 5 s after each addition.
3. Incubate for 15 min at RT.
4. Add the 250 µl transfection mixture drop-wise into each well (Note: for the 96-well format, the amount of transfection mixture added per well is only part of the total volume as indicated in Table 2).
5. Gently rock the plates back and forth and from side to side, and return the plate to the 37˚C CO2 incubator. It is not necessary to remove complexes or to change/add medium after transfection.
6. Analyze after incubating for 24 h or longer.
7. For stable transfection, start the antibiotic selection 24-48 hours after transfection. Grow cells in selective medium 10-15 days.

After you have completed the optimization step, choose the amount of Continuum™ that gives you the optimal balance of potency & low cytotoxicity. Use this volume for all future experiments on this specific cell type.

1.3 Scale Up or Down Transfection

Use Table 3 to scale the volumes for your transfection experiment.

Table 3. Scaling Up or Down Transfection Instruction

Culture Vessel	Multiplication Factor[1]	Vol. Complex DMEM per well (µl)	DNA (µg)	Continuum™ (µl)
96-well	0.17	10	0.05	0.04-0.2
48-well	0.50	25	0.125	0.1-0.5
24-well	**1.00**	**50**	**0.25**	**0.2-1.0**
12-well	2.00	100	0.5	0.4-2.0
6-well	5.00	200	1.25	1.0-5.0
60-mm	11.05	500	2.75	2.3-11.5
10-cm	28.95	1000	7	5.8-29
T75	39.47	1500	10	7.9-39

[1]After determining the optimum reagent amount, use the multiplication factor to determine the reagent amount needed for your new plate format.
[2]Optimum amount needed is determined from the protocol in the previous two pages.

1.4 Virus Production

Continuum™ is ideal for virus production, especially retrovirus, AAV and lentivirus, in adherent cells (ex: HEK-293T). For co-transfection of multiple plasmids, the total DNA amount per well/plate should not exceed the DNA amount indicated in Table 3. The ratio to use for each plasmid depends on the size of the plasmids, the plasmid constructs and the desired expression level of each plasmid. Please adjust the ratios according to your application. Each plasmid should represent at least 10% of the total DNA amount per well/plate. The following conditions are given per 100 mm dish for HEK-293T cells. If using other types of cells or other formats, optimize and scale according to Table 2 and Table 3.

1. Dilute 7 µg total DNA amount into 1000 µl regular high glucose DMEM without serum. Mix by vortexing.
2. Add 7-10 µl Continuum™, vortex for 10 s.
3. Incubate for 15 min at RT.
4. Add the transfection mix per dish drop wise onto the cells in the serum containing medium, and distribute evenly.
5. Gently rock the dish back and forth and from side to side.
6. Incubate in tissue culture incubator for 4h. Replace transfection medium with cell growth medium and return the dish to the incubator.
7. Incubate 24 to 72 h and proceed to virus purification and titration.

Continuum™ Transfection Reagent

1. siRNA Transfection

2.1 Cell Seeding: For optimal siRNA transfection conditions, we recommend using cells which are 50% confluent at the time of transfection. Typically, for experiments in 6-well plates, 100,000 to 150,000 cells are seeded per well in 2ml of growth medium without antibiotics 24 hours prior to transfection. For other culture formats, refer to Table 4.

Table 4. Recommended number of cells to seed the day before transfection in culture medium without antibiotics

Culture Vessel	Number of Adherent cells to seed	Surface area per well (cm^2)	Medium per well to seed the cells (ml)
24-well	25,000-40,000	1.9	0.5
12-well	50,000-80,000	3.8	1
6-well/35mm	100,000-150,000	9.4	2
60 mm/flask 25 cm^2	200,000-500,000	25-28	5
100 mm/flask 75 cm^2	$0.5x10^6$-$1x10^6$	75-78.5	10

2.2 siRNA Transfection: For optimal siRNA-mediated silencing, we recommend using 10 to 50 nM siRNA (final concentration). The following conditions are given per well of a 6-well plate. For other culture formats, please refer to Table 5.

1. Dilute 22 to 110 pmoles siRNA (final concentration: 10 to 50 nM) into 200µl of regular high glucose DMEM without serum. Mix by vortexing.
2. Briefly vortex Continuum™, and add 1.0 to 5.0 µl into the diluted siRNA. Immediately vortex for 10s.
3. Incubate for 15 min at RT.
4. Add the transfection mixture drop-wise into each well.
5. Gently rock the plates back and forth and from side to side, and return the plate to the 37˚C CO2 incubator.
6. Analyze after incubating for 24 h or longer.

Table 5. siRNA transfection guidelines according to the cell culture vessel per well.

Culture Vessel	siRNA (pmole) 10 nM	siRNA (pmole) 50 nM	Continuum™ (µl)	DMEM (µl)	Growth Medium (ml)	Final Volume in the well (ml)
24-well	5.5	27.5	*0.2-1.0	50	0.5	0.55
12-well	11	55	*0.4-2.0	100	1	1.1
6-well / 35 mm	22	110	*1.0-5.0	200	2	2.2
60 mm/ flask 25 cm^2	44	220	2.3-11.5	400	4	4.4
100 mm/ flask 75 cm^2	121	605	5.8-29.0	1100	11	12.1

**Dilute Continuum™ 1:5 with H_2O prior to application (4 µl reagent + 16 µl H_2O), and then use 5 times of the volume in the table for accurate pipetting.*