Simple Optimization guide in a 24-well format — Viromer® PLASMID

Storage
Store the Viromer® PLASMID kit at 4°C.

Before you start
Warm all reagents to room temperature.
Optional: Change media before transfection to remove dead cells.

Cell densitiy and confluency
Target: 60-80% at the time of transfection

Troubleshooting
In case of toxicity
Change media 4h after transfection. Use a lower amount of transfection complex on your cells. Use less Viromer® in step 2 of the protocol.

All cells are different so a good optimization is requested each time you start working with a new cell type or plasmid. We highly recommend to do the detailed optimization experiment on page 3, 5 or 7 at the beginning.

	96-well	48-well	24-well	12-well	6-well	10cm dish
medium / well	100µl	250µl	500µl	1ml	2ml	10ml
volumes used for preparing transfection complexes	1/5 x	½ x	standard	x2	x4	x20

You will find detailed optimization guides for 96-, 24- and 6-well transfections in the following pages. If you want to work in a different well-format, up- and downscaling is done easily by having a look at the volume of medium used in the different well sizes and adapt the given protocol volumes to the larger or smaller well size.

Standard Protocol

Step 1: **Preparation of pDNA**

Dilute your pDNA stock solution in provided Buffer PLASMID at 11 ng/µl. Prepare a volume of 330µl.

Step 2: **Preparation of Viromer®**

Place a **0,2µl** droplet Viromer® onto each wall of 4 fresh tubes. Immediately add **4,8µl** of Buffer PLASMID and vortex 3-5s.

Always add buffer to Viromer® and not vice versa!

Provide Viromer® into 4 fresh tubes.

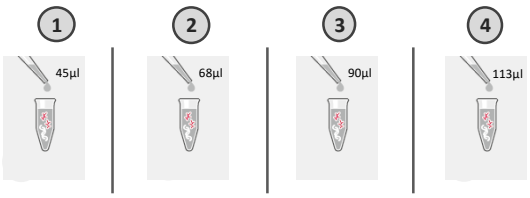
4x

Dilute Viromer® in buffer.

4x

Step 3: **Complexation**

Pipette the following volumes of pDNA (step 1) on the 5µl of Viromer® solution from step 2.



Mix swiftly and incubate 15min at room temperature.

Step 4: **Add 10µl of the transfection complexes on the cells**

pDNA per well: 100ng

Please perform triplicates or see next page for the detailed optimization guide!

Step 5: **Read-out**

Incubate cells as usual. There is no need to change medium unless high amounts of transfection complex cause toxicity.

Monitor effects 24-72 hours post-transfection and determine the best conditions for your special cells.

Detailed Optimization Guide in a 96-well Format — Viromer® PLASMID

Like we humans, all the cell types are different. A protocol which is working great in one cell type is not always transferable to a different cell type. That's why a good optimization is necessary to achieve the efficiencies you are looking for.

This is a more detailed optimization guide where you can do the most important steps in one experiment.

- playing on the amount of pDNA on the cells
- playing on the Viromer® – pDNA ratio for a better packing

It will help you to determine the optimal conditions for your special cells and plasmid much faster.

Step 1: pDNA 11ng/µl — provide 280µl

Step 2:

	tube 1	2	3	4	
Viromer® PLASMID in µl	0,24	0,2	0,16	0,12	**Always add buffer to Viromer®, not vice versa!** vortex 3-5s
Buffer pDNA in µl	5,76	4,8	3,84	2,88	
	54	67,5	72	67,5	

Step 3: **complexation**

Pipette x µl of your pDNA from step 1 into the 4 tubes with diluted Viromer® of step 2. Mix swiftly and incubate 15min at room temperature.

Step 4:

Transfer x µl of complexes into the wells according to the pipetting scheme.

Forward transfection: Add transfection complexes onto the cells seeded a day before. Mix carefully.

Reverse Transfection: Add transfection complexes to empty wells and seed the cells (in 100µl) immediately afterwards. Mix carefully.

96-well	1	2	3	4	5	6
tube 1	2µl	5µl	10µl	15µl	20µl	
tube 2	2µl	5µl	10µl	15µl	20µl	
tube 3	2µl	5µl	10µl	15µl	20µl	
tube 4	2µl	5µl	10µl	15µl	20µl	
	20ng DNA/well	50ng DNA/well	100ng DNA/well	150ng DNA/well	200ng DNA/well	

playing on the Viromer® - pDNA ratio

playing on the amount of complexes / pDNA on the cells

Step 5: **Read-out**

Incubate cells as usual. There is no need to change medium unless high amounts of transfection complex cause toxicity.
Monitor effects 24-72 hours post-transfection and determine the best conditions for your special cells.

In any case of questions regarding transfection or optimization, feel free to contact us at info@lipocalyx.de and discuss your experiment with us.

Standard Protocol

Step 1: **Preparation of pDNA**

Dilute your pDNA stock solution in provided Buffer PLASMID at 11 ng/µl. Prepare a volume of 330µl.

Step 2: **Preparation of Viromer®**

Place a **0,2µl** droplet Viromer® onto each wall of 4 fresh tubes. Immediately add **4,8µl** of Buffer PLASMID and vortex 3-5s.

Always add buffer to Viromer® and not vice versa!

Provide Viromer® into 4 fresh tubes.

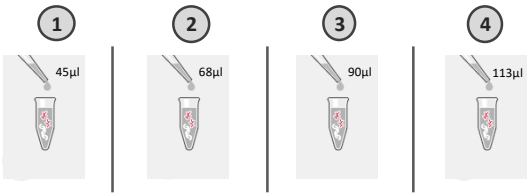
4x

Dilute Viromer® in buffer.

4x

Step 3: **Complexation**

Pipette the following volumes of pDNA (step 1) on the 5µl of Viromer® solution from step 2.



Mix swiftly and incubate 15min at room temperature.

Step 4: **Add 50µl of the transfection complexes on the cells**

pDNA per well: 500ng

Please look also at the next page for the detailed optimization guide!

Step 5: **Read-out**

Incubate cells as usual. There is no need to change medium unless high amounts of transfection complex cause toxicity.

Monitor effects 24-72 hours post-transfection and determine the best conditions for your special cells.

Detailed Optimization Guide in a 24-well Format — Viromer® PLASMID

Like we humans, all the cell types are different. A protocol which is working great in one cell type is not always transferable to a different cell type. That's why a good optimization is necessary to achieve the efficiencies you are looking for.

This is a more detailed optimization guide where you can do the most important steps in one experiment.

- playing on the amount of pDNA on the cells
- playing on the Viromer® – pDNA ratio for a better packing

It will help you to determine the optimal conditions for your special cells and plasmid much faster.

Step 1: pDNA 11ng/µl — provide 1200µl

Step 2:

	tube 1	2	3	4	
Viromer® PLASMID in µl	1,2	0,8	0,6	0,6	**Always add buffer to Viromer®, not vice versa!** vortex 3-5s
Buffer pDNA in µl	28,8	19,2	14,4	14,4	
	270	272	270	339	

Step 3: **complexation**

Pipette x µl of your pDNA from step 1 into the 4 tubes with diluted Viromer® of step 2. Mix swiftly and incubate 15min at room temperature.

Step 4:

Transfer x µl of complexes into the wells according to the pipetting scheme.

Forward transfection: Add transfection complexes onto the cells seeded a day before. Mix carefully.

Reverse Transfection: Add transfection complexes to empty wells and seed the cells (in 500µl) immediately afterwards. Mix carefully.

24-well	1	2	3	4	5	6
tube 1	10µl	25µl	50µl	75µl	100µl	
tube 2	10µl	25µl	50µl	75µl	100µl	
tube 3	10µl	25µl	50µl	75µl	100µl	
tube 4	10µl	25µl	50µl	75µl	100µl	
	100ng DNA/well	250ng DNA/well	500ng DNA/well	750ng DNA/well	1000ng DNA/well	

playing on the Viromer® - pDNA ratio

playing on the amount of complexes / pDNA on the cells

Step 5: **Read-out**

Incubate cells as usual. There is no need to change medium unless high amounts of transfection complex cause toxicity.
Monitor effects 24-72 hours post-transfection and determine the best conditions for your special cells.

In any case of questions regarding transfection or optimization, feel free to contact us at info@lipocalyx.de and discuss your experiment with us.

Standard Protocol

Step 1: **Preparation of pDNA**

Dilute your pDNA stock solution in provided Buffer PLASMID at 11 ng/µl. Prepare a volume of 1300µl.

Step 2: **Preparation of Viromer®**

Place a **0,8µl** droplet Viromer® onto each wall of 4 fresh tubes. Immediately add **19,2µl** of Buffer PLASMID and vortex 3-5s.

Always add buffer to Viromer® and not vice versa!

Provide Viromer® into 4 fresh tubes.

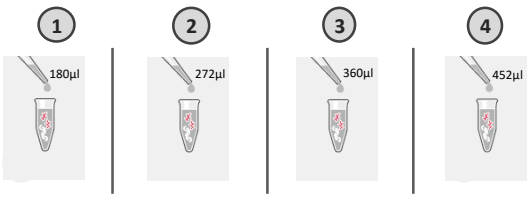
4x

Dilute Viromer® in buffer.

4x

Step 3: **Complexation**

Pipette the following volumes of pDNA (step 1) on the 5µl of Viromer® solution from step 2.



Mix swiftly and incubate 15min at room temperature.

Step 4: **Add 200µl of the transfection complexes on the cells**

pDNA per well: 2µg

Please look also at the next page for the detailed optimization guide!

Step 5: **Read-out**

Incubate cells as usual. There is no need to change medium unless high amounts of transfection complex cause toxicity.

Monitor effects 24-72 hours post-transfection and determine the best conditions for your special cells.

Detailed Optimization Guide in a 6-well Format — Viromer® PLASMID

Like we humans, all the cell types are different. A protocol which is working great in one cell type is not always transferable to a different cell type. That's why a good optimization is necessary to achieve the efficiencies you are looking for.

This is a more detailed optimization guide where you can do the most important steps in one experiment.

- playing on the amount of pDNA on the cells
- playing on the Viromer® – pDNA ratio for a better packing

It will help you to determine the optimal conditions for your special cells and plasmid much faster.

Step 1: pDNA 11ng/µl — provide 4800µl

Step 2:

	tube 1	2	3	4	
Viromer® PLASMID in µl	4,8	3,2	2,4	2,4	**Always add buffer to Viromer®, not vice versa!** vortex 3-5s
Buffer pDNA in µl	115,2	76,8	57,6	57,6	
	1080	1088	1080	1080	

Step 3: **complexation**

Pipette x µl of your pDNA from step 1 into the 4 tubes with diluted Viromer® of step 2. Mix swiftly and incubate 15min at room temperature.

Step 4:

Transfer x µl of complexes into the wells according to the pipetting scheme.

Forward transfection: Add transfection complexes onto the cells seeded a day before. Mix carefully.

Reverse Transfection: Add transfection complexes to empty wells and seed the cells (in 2ml) immediately afterwards. Mix carefully.

6-well	1	2	3	4	5	6
tube 1	40µl	100µl	200µl	300µl	400µl	
tube 2	40µl	100µl	200µl	300µl	400µl	
tube 3	40µl	100µl	200µl	300µl	400µl	
tube 4	40µl	100µl	200µl	300µl	400µl	
	400ng DNA/well	1µg DNA/well	2µg DNA/well	3µg DNA/well	4µg DNA/well	

playing on the Viromer® - pDNA ratio

playing on the amount of complexes / pDNA on the cells

Step 5: **Read-out**

Incubate cells as usual. There is no need to change medium unless high amounts of transfection complex cause toxicity.
Monitor effects 24-72 hours post-transfection and determine the best conditions for your special cells.

In any case of questions regarding transfection or optimization, feel free to contact us at info@lipocalyx.de and discuss your experiment with us.