# Optimization Guide — Viromer® mRNA


## Storage
Store the Viromer® mRNA 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. Note that mRNA expression is much stronger compared to plasmids!
Use less Viromer® in step 2 of the protocol.

## Content and formats

| | | |
|---|---|---|
| **Viromer® mRNA** | 100 transfections | VmR-01LB-00 |
| Incl. Buffer mRNA | 900 transfections | VmR-01LB-01 |
| | 3 x 900 transfections | VmR-01LB-03 |

## Standard Protocol

well format

| | | 96-well | 24-well | 6-well | |
|---|---|---|---|---|---|
| Step 1: | mRNA (11ng/µl) in µl | 9 | 45 | 180 | |
| Step 2: | Viromer® mRNA in µl | 0,04 | 0,2 | 0,8 | **Always add buffer to Viromer®, not vice versa!** vortex 3-5s |
| | Buffer mRNA in µl | 0,96 | 4,8 | 19,2 | |
| Step 3: | **complexation** | Pipette the mRNA from step 1 into the diluted Viromer® of step 2. Mix swiftly and incubate 15 min at room temperature. | | | |
| Step 4: | Transfer x µl of complexes into the wells. | 10 µl<br>100 ng/well | 50 µl<br>500 ng/well | 200 µl<br>2 µg / well | |

**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 (100µl) immediately afterwards. Mix carefully.

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 12-24 hours post-transfection and determine the best conditions for your special cells.
Note: mRNA expression can start as early as 6h after transfection.

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

# Detailed Optimization Guide in a 96-well Format **Viromer® mRNA**

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 mRNA on the cells
- playing on the Viromer® – mRNA ratio for a better packing

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

**Step 1:** mRNA 11ng/µl — provide 250µl

**Step 2:**

| | tube 1 standard | 2 1,5x | 3 2x | 4 2,5x | |
|---|---|---|---|---|---|
| Viromer® mRNA in µl | 0,25 | 0,38 | 0,5 | 0,63 | **Always add buffer to Viromer®, not vice versa!** vortex 3-5s |
| Buffer mRNA in µl | 6 | 6 | 6 | 6 | |
| | 56,25 | 56,25 | 56,25 | 56,25 | |

**Step 3:** **complexation**

Pipette x µl of your mRNA 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.

| 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 mRNA/well | 250ng mRNA/well | 500ng mRNA/well | 750ng mRNA/well | 1000ng mRNA/well | |

playing on the Viromer® - mRNA ratio

playing on the amount of complexes / mRNA 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 12-24 hours post-transfection and determine the best conditions for your special cells.
Note: mRNA expression can start as early as 6h after transfection.

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

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

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 mRNA on the cells
- playing on the Viromer® – mRNA ratio for a better packing

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

**Step 1:** mRNA 11ng/µl — provide 1400µl

**Step 2:**

| | tube 1 standard | tube 2 1,5x | tube 3 2x | tube 4 2,5x |
|---|---|---|---|---|
| Viromer® mRNA in µl | 1,5 | 2,25 | 3,0 | 3,75 |
| Buffer mRNA in µl | 36 | 36 | 36 | 36 |
| | 337,5 | 337,5 | 337,5 | 337,5 |

**Always add buffer to Viromer®, not vice versa!**
vortex 3-5s

**Step 3:** **complexation**

Pipette x µl of your mRNA 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 mRNA/well | 250ng mRNA/well | 500ng mRNA/well | 750ng mRNA/well | 1000ng mRNA/well | |

playing on the Viromer® - mRNA ratio

playing on the amount of complexes / mRNA 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 12-24 hours post-transfection and determine the best conditions for your special cells.
Note: mRNA expression can start as early as 6h after transfection.

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