LabBOOK

VIROMER® RED
VIROMER® YELLOW

plasmid DNA/mRNA transfection

General information

Technology

Viromer® RED and **Viromer® YELLOW** are polymer-based transfection reagents featuring a viral mechanism of membrane fusion. They do form transfection complexes with plasmid DNA (pDNA) or messenger RNA (mRNA), which are taken up by endocytosis, a process that involves the formation of an acidic compartment. The low pH in late endosomes acts as a chemical switch that renders the Viromer® surface hydrophobic and facilitates membrane crossing. This "Active Endosome Escape" technology is safe and maximizes transfection efficiency as it is using a natural uptake pathway.

Key Benefits

+ Active Escape Technology	> Efficacy and safety during uptake
+ Zero Charge	> Fully compatible with serum or antibiotics. Fully compatible with suspension cells.
+ Stable Particles	> Reproducible results
+ Lipid free	> Works in adipocytes
+ Reverse Transfection	> Ready for High-Throughput Screening

Application

Viromer® RED and **Viromer® YELLOW** are optimized for *in vitro* transfection of pDNA and mRNA. They are highly effective on a wide range of standard and hard-to-transfect cells including suspension cells, stem cells and primary cells. For the transfection of siRNA and miRNA, please refer to **Viromer® BLUE** and **Viromer® GREEN**.

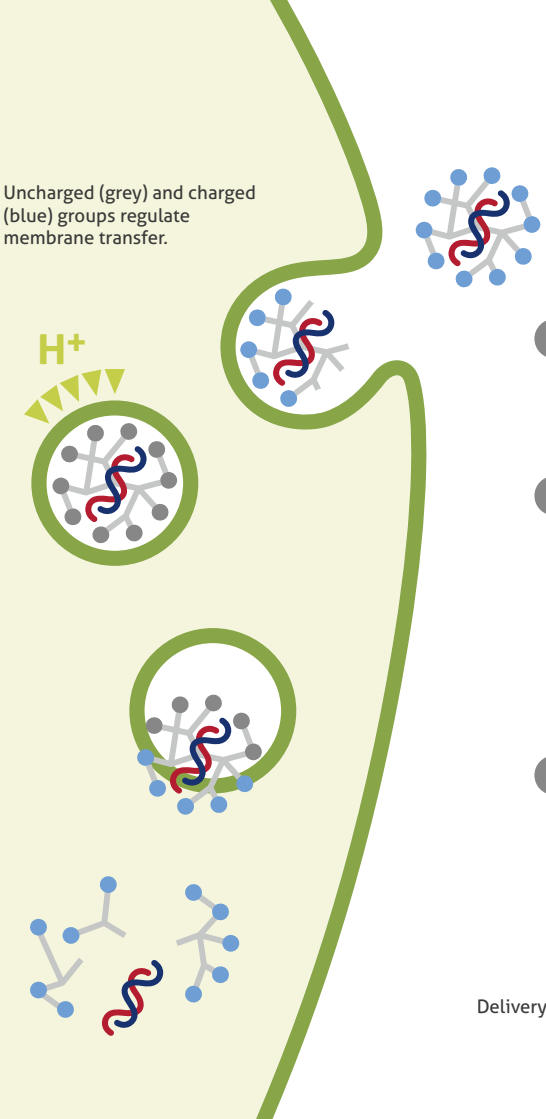
Viromer® RED or Viromer® YELLOW?

The two transfection reagents differ in their surface and backbone chemistry but follow the same workflow.

- **Viromer® RED** > is a versatile standard reagent
- **Viromer® YELLOW** > is more selective for particular cells

A list of cell types and transfection results is available at:
https://viromer-transfection.com/data-by-cell-type/cell-transfection-a-z
If the cell type of your interest is not listed, parallel tests with both **Viromer® RED** and **Viromer® YELLOW** are recommended.

The Viromer® Uptake Pathway: The Active Escape Process



Step 1

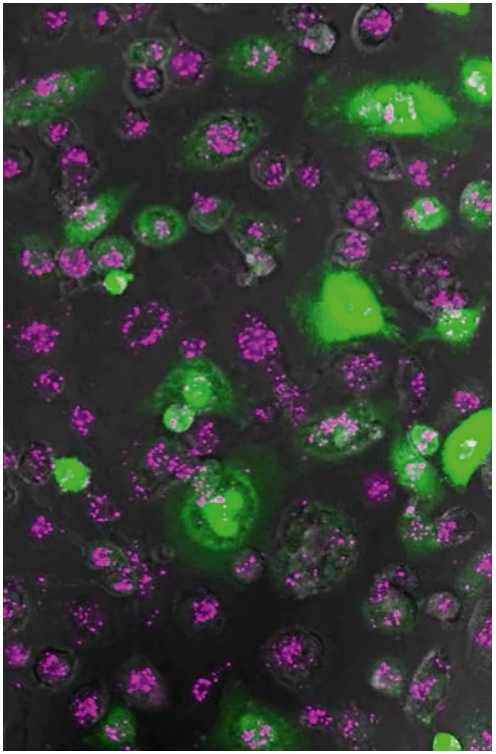
The Viromer®: DNA (or -mRNA) complexes are taken up in endosomes

Step 2

Active Escape. Endosomes acidify which in turn provides membrane-penetrating properties to the Viromers. The transfection complex exits from the lysosomal degradation pathway into cytosol.

Step 3

In the cytosol, Viromers regain charge so that no back-transport occurs. The DNA (or -mRNA) dissociates from the transfection complex.



Delivery of Cy3b-tagged (magenta) GFP-encoding mRNA into human monocytes-derived macrophages using **Viromer® RED**
D. Russel, Cornell University, USA

Protocol Guidelines

Material required

Use a sterile workplace and materials as required for any cell culture work. Warm all reagents to room temperature. Prepare fresh transfection complexes each time you use the products. **Buffer RED** and **Buffer YELLOW** (provided with the kits) are required to make dilutions of Viromer® reagents and pDNA/mRNA.

Cell Culture and Plating

Viromer® **RED** and Viromer® **YELLOW** are compatible with standard cell culture media, serum and antibiotics.

- Seed the cells in complete medium the day before transfection
- Adjust the cell density so that 60 - 80% confluency are reached at the time of transfection
- For experiments lasting for more than 48h, replenish medium before starting transfection.

Note: The preparation of suspension cells might also be achieved few hours before transfection.

Recommended number of cells to be seeded one day before transfection (for standard cells, read-out 24h - 48h post-transfection)

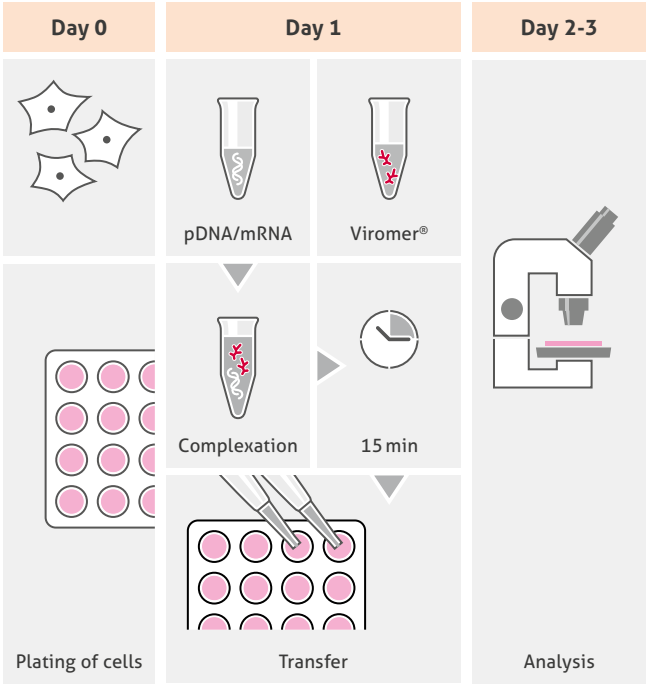
Multiwell plate type	96-well	24-well	6-well
Adherent cells			
Cells seeded per well	12,000	60,000	250,000
*Range	± 3,000	± 20,000	± 80,000
Suspension cells			
Cells seeded per well	48,000	240,000	1,000,000
*Range	± 12,000	± 80,000	± 320,000
Medium per well	0.1 ml	0.5 ml	2 ml

* In reverse transfection protocols, cell numbers should be on the higher end.

The optimum density for transfection is highly variable among cell types and needs to be determined empirically. Please refer to optimization on page 12 for more details.

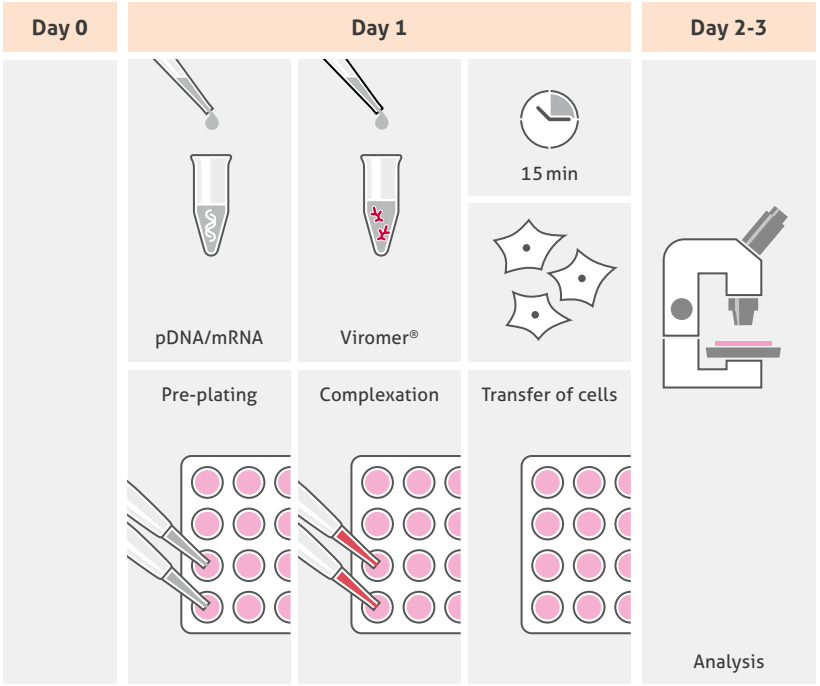
Transfection workflow

We here describe a common workflow for the Forward Transfection of adherent or suspension cells using **Viromer® RED** or **Viromer® YELLOW**.



For Reverse Transfection and high-throughput screening (HTS) applications, the use of **Viromer® RED** or **Viromer® YELLOW** is based on a common protocol in which all steps - complexation, transfer in wells and adding of cells - are performed at the same day.

Please contact the Viromer® technical support for detailed information.



General Tips for Transfection at the bench!

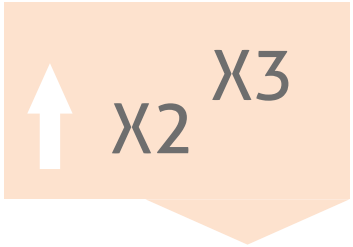
Add extra volume

All protocols given here are based on exact volumes for single-well transfections. Whenever possible, plan for larger experiments including replicates and prepare a master mix. Work with an extra 10% of all volumes to account for pipetting errors.

Use minimum volumes

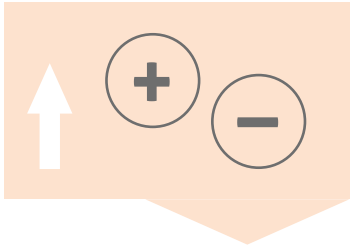
We advise to prepare at least 50µl of transfection complex to ensure homogenous complexation and to limit variability.

Replicates



As for any other bench work involving cells in culture or preparation of very small volumes, plan for replicates to create a solid base of data for the interpretation of results and related statistical analysis.

Controls



To verify efficient transfection, use a positive control such as a luciferase or a GFP plasmid (or mRNA encoding luciferase or GFP).

As negative control, use wells with untreated cells.

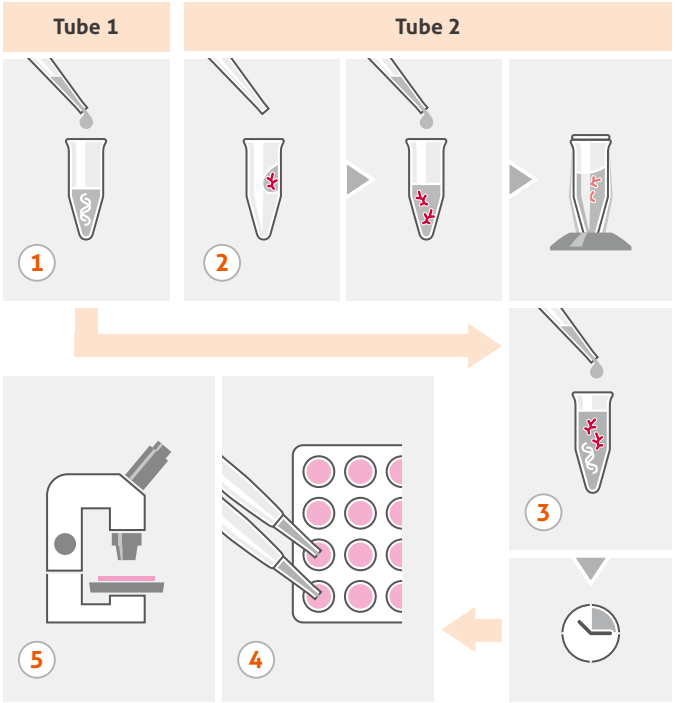
Basic Transfection Protocol for **Adherent cells**

1. **Preparation of pDNA/mRNA** » Tube 1
   - Dilute your pDNA/mRNA stock solution in provided buffer at **11 ng/µl**.
   - Prepare a volume of **45 µl**.
2. **Preparation of Viromer®** » Tube 2
   - Place a **0.2-µl** droplet of Viromer® onto the wall of a fresh tube
   - Immediately add **4.8 µl** of buffer and vortex 3-5s
     Always add buffer to Viromer®, not vice versa!
3. **Complexation** » Tube 1 –> Tube 2
   - Pipette **45 µl** of the pDNA/mRNA solution from Tube 1 on the **5 µl** of Viromer® solution in Tube 2.
   - Mix swiftly and incubate 15 min at room temperature.
4. **Add 50µl of the transfection complex on the cells**
5. **Read-out**
   - Incubate cells as usual. There is no need to change medium unless high amounts of transfection complex cause toxicity.
   - For pDNA, monitor effects 24-72 hours post-transfection.
   - For mRNA, expression can start as early as 6 hours post-transfection.

Corresponding volumes in other culture formats

Multiwell plate type	96-well	24-well	6-well
Tube 1	9 µl	45 µl	180 µl
Tube 2	0.04 µl Viromer® 0.96 µl buffer	0.2 µl Viromer® 4.8 µl buffer	0.8 µl Viromer® 19.2 µl buffer
Transfer volume	10 µl	50 µl	200 µl
DNA/mRNA on cells	100 ng	500 ng	2 µg

24-well format | 500 ng pDNA or mRNA on cells (≈ 1ng/µl)



Note: All volumes are given as exact volumes for single-well transfections.

Basic Transfection Protocol for **Suspension cells**

1 Preparation of pDNA/mRNA » Tube 1

- Dilute your pDNA/mRNA stock solution in provided buffer at **11 ng/µl**.
- Prepare a volume of **90 µl**.

2 Preparation of Viromer® » Tube 2

- Place a **0.4-µl** droplet of Viromer® onto the wall of a fresh tube
- Immediately add **9.6 µl** of buffer and vortex 3-5 s
  Always add buffer to Viromer®, not vice versa!

3 Complexation » Tube 1 –> Tube 2

- Pipette **90 µl** of the pDNA/mRNA solution from Tube 1 on the **10 µl** of Viromer® solution in Tube 2.
- Mix swiftly and incubate 15 min at room temperature.

4 Add 100 µl of the transfection complex on the cells

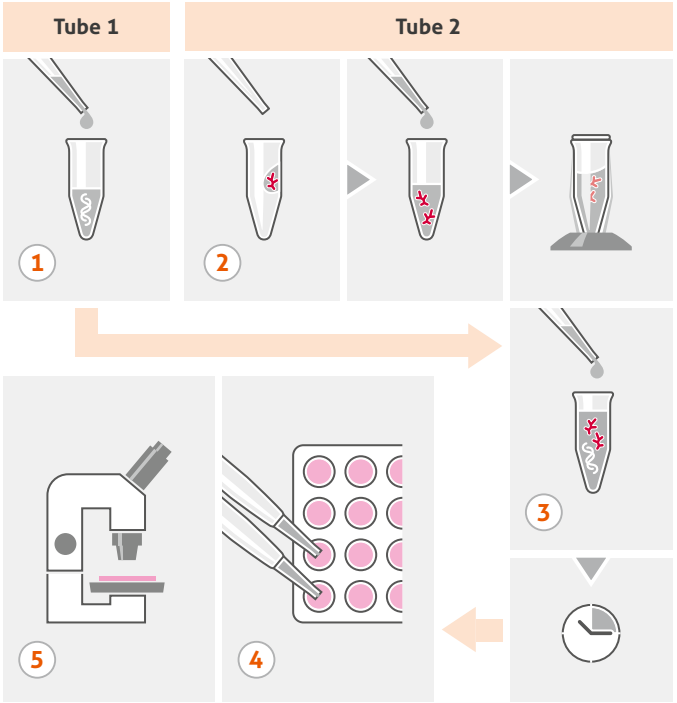
5 Read-out

- Incubate cells as usual. There is no need to change medium unless high amounts of transfection complex cause toxicity.
- For pDNA, monitor effects 24-72 hours post-transfection.
- For mRNA, expression can start as early as 6 hours post-transfection.

Corresponding volumes in other culture formats

Multiwell plate type	96-well	24-well	6-well
Tube 1	18 µl	90 µl	360 µl
Tube 2	0.08 µl Viromer® 1.92 µl buffer	0.4 µl Viromer® 9.6 µl buffer	1.6 µl Viromer® 38.4 µl buffer
Transfer volume	20 µl	100 µl	400 µl
DNA/mRNA on cells	200 ng	2 µg	4 µg

24-well format | 2 µg pDNA/mRNA on cells (≈1.65ng/µl)



Note: All volumes are given as exact volumes for single-well transfections.

Transfection optimization

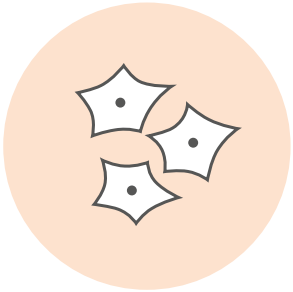
You do see efficacy, but your protein expression is too low or there is toxicity?

Except for cases where the basic protocol is absolutely right, we suggest reproducing the initial findings and creating stronger data with a few optimization steps.
Cell density, amount of transfection complex, Viromer®-pDNA (or -mRNA) ratio, and duration of transfection (incubation time) are the determining factors for protocol optimization. For any new combination of cells, plasmid and DNA, or mRNA, we strongly recommend adjusting each parameter one by one with the following guidelines.

1. Amount of transfection complex

Test increasing amounts of transfection complex arriving onto the cells

Maximize effect with a fixed Viromer®-pDNA (or -mRNA) ratio



2. Cell density & confluency

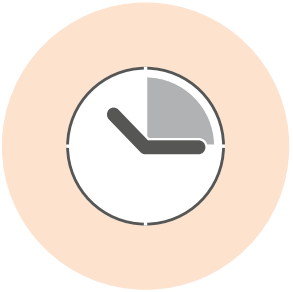
Growth, cell division or the existence of cell contacts regulate uptake of materials

Cell density is a strong regulator of transfection effects

3. Viromer® -pDNA (or -mRNA) ratio

Higher concentration of Viromer® results in better transfection.

Maximize efficacy at low concentrations of DNA or mRNA



4. Time for analysis

24-72h for pDNA , as early as 6h for mRNA. Change of medium in case of toxicity.

The perfect timing for optimal read-outs

Amount of Viromer® -pDNA (or -mRNA) transfection complex

Five-step titration to determine the lowest pDNA/mRNA amount that could be used.

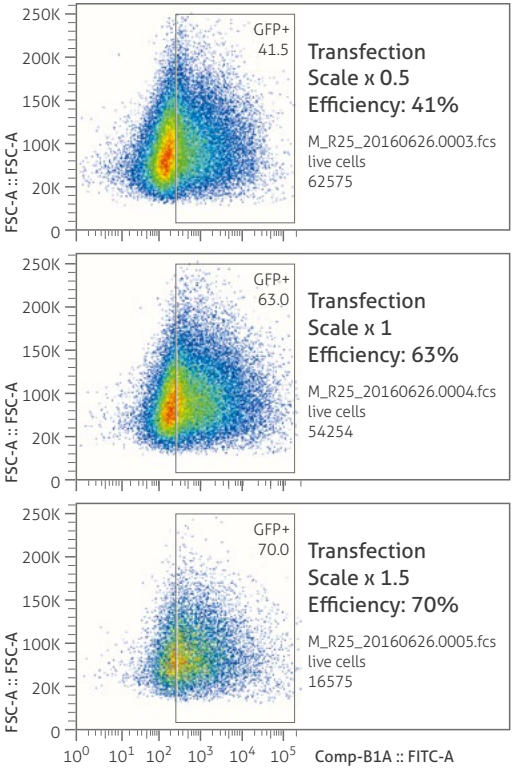
		96-well	24-well	6-well
1	• Dilute pDNA or mRNA to 11ng/µl using provided buffer. – Tube 1			
	Volume needed for step 3	56.25 µl	337.5 µl	1125 µl
2	• Add buffer on Viromer® for dilution (not vice versa!) – Tube 2			
	Viromer®	0.25 µl	1.5 µl	5 µl
	Buffer	6 µl	36 µl	120 µl
3	• Combine Viromer® and pDNA/mRNA. Incubate the master mix for 15 min – Tube 1 >> Tube 2			
	• Tritate the transfection complex on cells using 5 different transfer volumes			

	Transfection Scale	DNA/mRNA per well		DNA/mRNA per well		DNA/mRNA per well	
4	0.2 x	2 µl	20 ng	10 µl	100 ng	40 µl	400 ng
	0.5 x	5 µl	50 ng	25 µl	250 ng	100 µl	1 µg
	1.0 x Standard	10 µl	100 ng	50 µl	500 ng	200 µl	2µg
	1.5 x	15 µl	150 ng	75 µl	750 ng	300 µl	3 µg
	2 x	20 µl	200 ng	100 µl	1000 ng	400 µl	4 µg

5 • Monitor effects 24-72h after transfection, as early as 6h for mRNA.

Note: Please, consider replicates and use of proper controls in parallel.

Adherent cells



Increasing efficiency of pDNA transfection in MCF-10A cells using **Viromer® RED** at three different transfection scales.

FACS data from E. Hadadi, ES Team-INSERM U935, Paris (France).

Amount of Viromer® -pDNA (or -mRNA) transfection complex

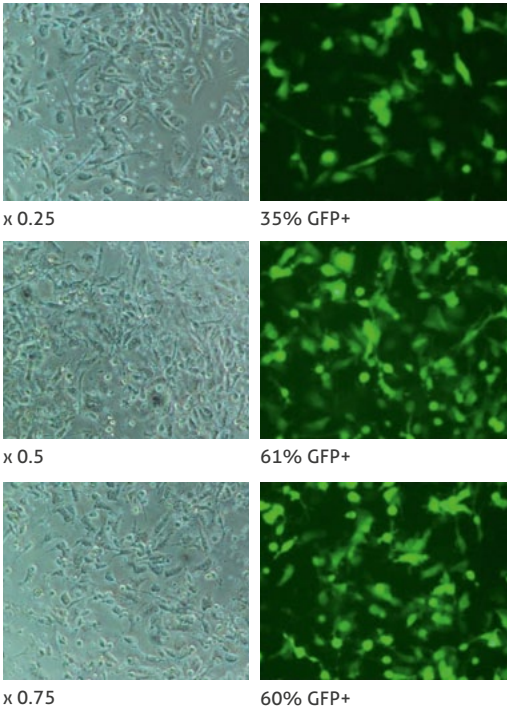
Five-step titration to determine the lowest pDNA/mRNA amount that could be used.

		96-well	24-well	6-well
1	• Dilute pDNA or mRNA to 11ng/µl using provided buffer. – Tube 1			
	Volume needed for step 3	90 µl	450 µl	1800 µl
2	• Add buffer on Viromer® for dilution (not vice versa!) – Tube 2			
	Viromer®	0.4 µl	2 µl	8 µl
	Buffer	9.6 µl	48 µl	192 µl
3	• Combine Viromer® and pDNA/mRNA. Incubate the master mix for 15 min – Tube 1 >> Tube 2			
	• Tritate the transfection complex on cells using 5 different transfer volumes			

	Transfection Scale	DNA/mRNA per well		DNA/mRNA per well		DNA/mRNA per well	
4	0.25 x	5 µl	50 ng	25 µl	250 ng	100 µl	1 µg
	0.5 x	10 µl	100 ng	50 µl	500 ng	200 µl	2 µg
	0.75 x	15 µl	150 ng	75 µl	750 ng	300 µl	3 µg
	1.0 x Standard	20 µl	200 ng	100 µl	1000 ng	400 µl	4 µg
	1.25 x	25 µl	250 ng	125 µl	1250 ng	500 µl	5 µg
5	• Monitor effects 24-72h after transfection, as early as 6h for mRNA.						

Note: Please, consider replicates and use of proper controls in parallel.

Suspension cells



Efficiency of mRNA transfection in human monocyte-derived dendritic cells using **Viromer® RED** at three different transfection scales.

Microscopy data from F. Gueugnon, Vaxeal Research, CEA Saclay (France).

Cell density and confluency

For each cell type, an optimum cell density for transfection needs to be determined.

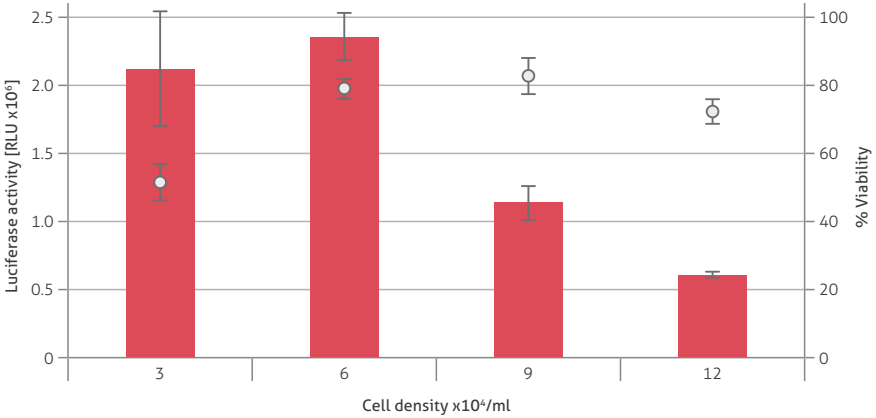
› Target: **60-80%** at the time of transfection

› Usual recommendations for common cells: seed the cells one day prior transfection at
- **8-15 x 10⁴ cells/ml for adherent cells,**
- **36-60 x 10⁴ cells/ml for suspension cells**

› Take into account
- cell specifics for adjustments e.g. growth rate, size, cell junctions
- selected assays and time-point requirements e.g., for short-term assays, plate cells at higher density than for long-term assays.

› Once optimized, keep parameters constant to ensure reproducibility in further experiments

C2C12 mouse myoblasts (undifferentiated)



Comparison of luciferase activity and cell viability 24h post-transfection of pDNA (6kb) with Viromer® RED at 4 different seeding densities
- Basic standard protocol in 96-well plate (n=6).

» The observed optimal density (6x10⁴ cells/ml) is under the recommended range, as C2C12 are large cells, form monolayers, and have a rapid growth (doubling time ≈12h)

Seed cells at ...

Recommended range
- Adherent cells: 8-15 x 10⁴ cells/ml
- Suspension cells: 36-60 x 10⁴ cells/ml

Start at lower density,

- Large cells
- High doubling rate
- Cells with tight junctions
- Read-out>48h

or high density.

- Small cells
- Low cell growth
- Difficulty to reach confluency
- Read-out <24-48h
- Reverse transfection

Analyse cells at ...

60–80% confluency

VIROMER® RED
VIROMER® YELLOW

VIROMER® -pDNA (or -mRNA) ratio

If no toxicity is observed, transfection efficiency can be enhanced by using more **Viromer®** reagent for a fixed amount of DNA or mRNA. We suggest adding more Viromer® reagent (x2, x2.5, x3...) at the dilution step (step 2 of the protocol) while keeping all other volumes constant.

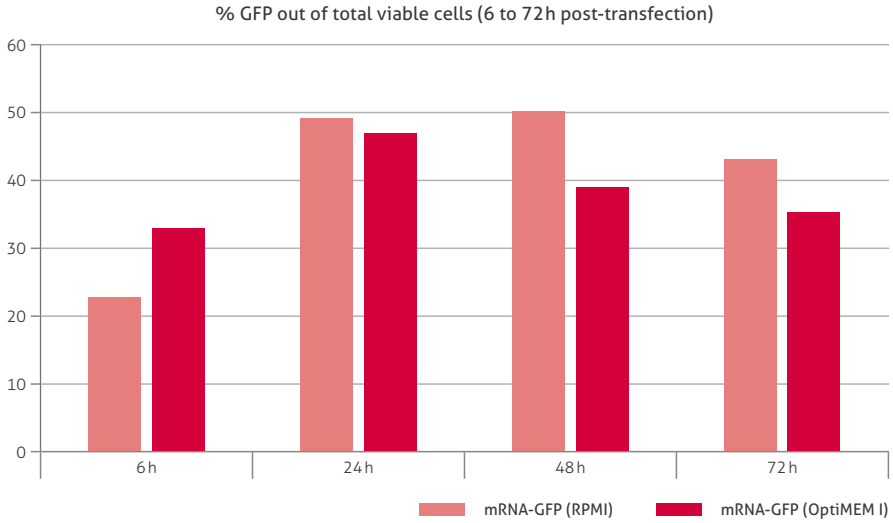
Inversely, if transfection with standard conditions is toxic to the cells, a lower ratio should be tested.

Time analysis

Maximum effects of transfected DNA are usually observed 24-72 hours post-transfection. Note that with mRNA, effects can be detected as early as 2 hours.

It is recommended to change the medium 4 hours after transfection in case of toxicity to the cells.

Optionally, supplementation or replacement of medium can be done 24h or 48h after transfection.



Optimization of read-out endpoint and use of different media for mRNA transfection in THP-1 monocytes with **Viromer® RED**. Transfection of GFP encoding mRNA in cells seeded in RPMI (10% FCS) or OptiMEM® medium (0% FCS), cell counting 6-72h post-transfection.

FACS data from S. Fabb, Monash Institute of Pharmaceutical Sciences, Victoria (Australia)

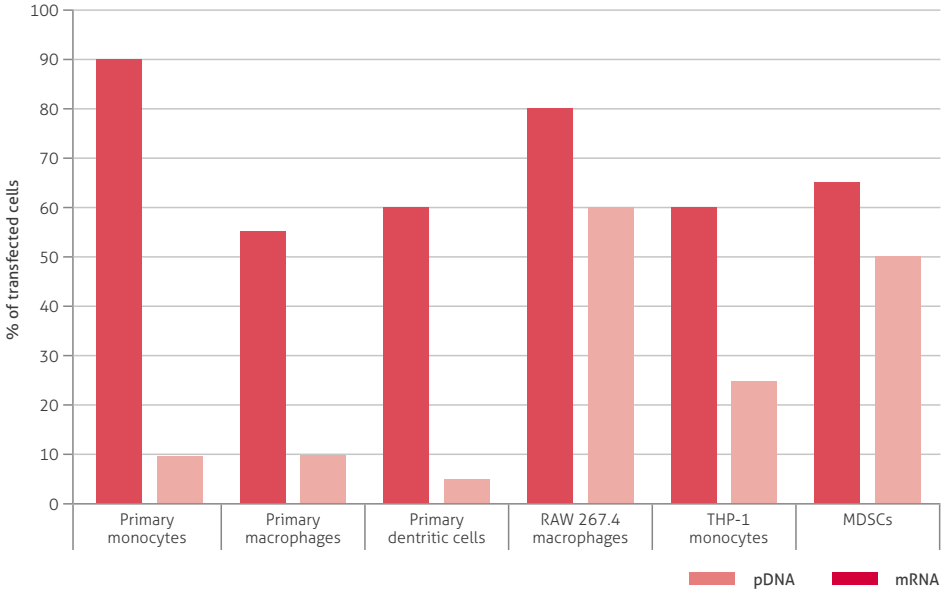
Special recommendations for mRNA transfection

Transfecting cells with mRNA sequences rather than plasmid DNA constructs gives a great chance to significantly increase protein expression levels. After delivery, mRNA is directly expressed in the cytosol through a promoter-independent process and protein is detectable as early as 6h post-transfection.

Viromer® RED and **Viromer® YELLOW** have been optimized to work equally strong with DNA and mRNA. Recent investigations have shown a clear advantage of mRNA transfection for some specific cells known as "resistant" to plasmid transfection:

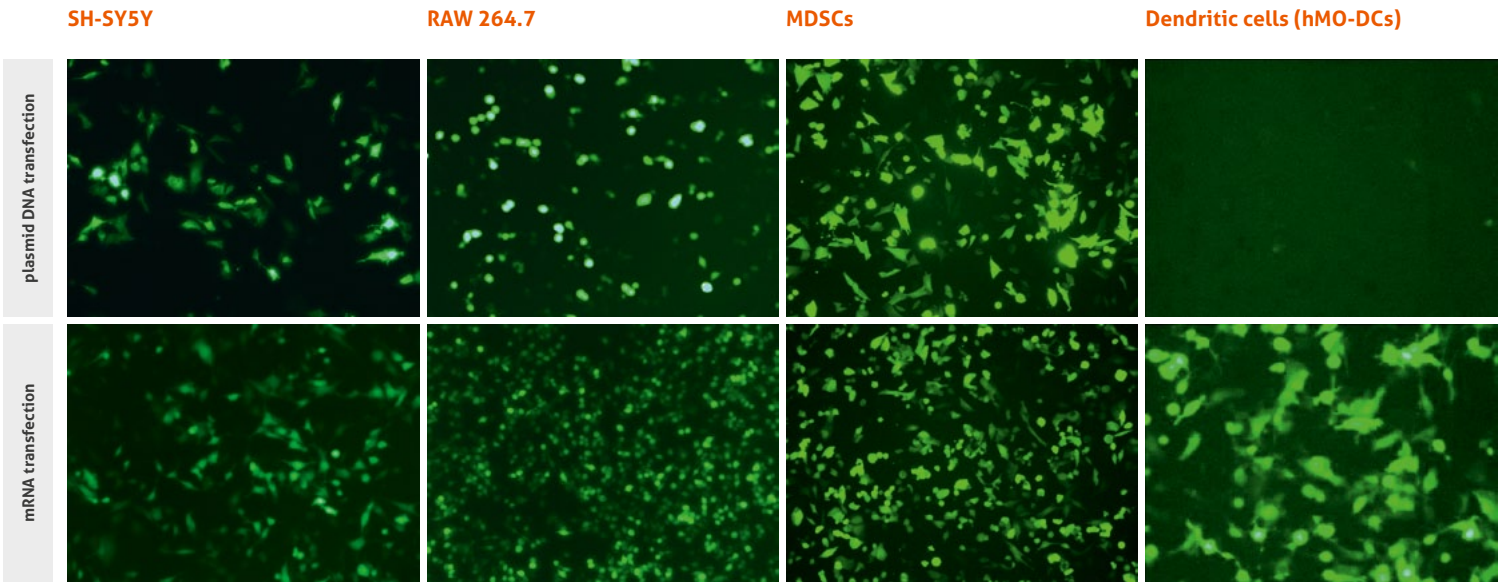
- cells with low division rate, e.g. primary neurons, differentiated skeletal muscle cells, and
- cells with cytosolic defense mechanisms against foreign DNA (innate immunity), e.g. AIM2-Interleukin or cGAS-Interferon enzymatic cascades of macrophages and monocytes.

IMPORTANTE NOTE: To produce stable and high quality mRNA for transfection and subsequent translation, it is recommended to use *in vitro* transcription commercial kits enabling 5´ capping and 3´ polyadenylation. Transcribed mRNA should be then purified.



Comparative transfection efficiency of **Viromer® RED** used for plasmid DNA or mRNA delivery in diverse immune cell lines (maximal percentage of positive cells reported by users).

To compare transfection efficiency of the Viromer® technology with both cargoes, we suggest using the **Start Positive® Controls**. (see details on page 16)



Data from H. Cynis, Fraunhofer Institute for Cell Therapy and Immunology, Halle, Germany

Data from F. Combes, Faculty of Veterinary Medicine, Ghent University, Belgium

Data from F. Gueugnon, Vaxeal Research, CEA-Saclay, France

Transfection using Start Positive® Controls.

Challenging cell lines were transfected with pCMV-GFP plasmid and GFP-mRNA using Start Positive® controls of **Viromer® RED**. Transfection was monitored using fluorescence microscopy. Between pDNA and mRNA we typically observe faster, homogeneous and stronger expression from mRNA. We attribute this to the instant availability of the transcript.

Trouble-shooting / Minimizing Background

Low transfection efficiency or no signal

First, follow recommendations for protocol optimization given previously:

- Use higher amount of transfection complex onto the cells, either by increasing pDNA/mRNA final amount or by seeding less cells per well
- Use higher amount of Viromer® in step 2 of the protocol
- Monitor effects at various time points from 6h to 96h post-transfection
- Ensure that the cell density is adequate

Additional tips:

- Check that Viromer® reagents were stored and used correctly.
- Transfection complexes should be prepared freshly.
- All materials should be sterile and DNase/RNase free.
- Control plasmid DNA constructs or mRNA sequences (e.g. high purity)

If you work with plasmid DNA and transfection efficiency is too low, consider the option of transfecting the corresponding transcribed mRNA (see previous recommendations).

Cell toxicity

- Change the medium 4h after transfection
- Use a lower amount of transfection complex on your cells
- Use less Viromer® in step 2 of the protocol

Use the Viromer® Start Positive® Controls!

Positive® Controls are pre-formulated Viromer® transfection complexes. Use these materials for evaluating transfection of new cell types with the Viromer® technology or as reference material, or to compare plasmid DNA and mRNA transfections.

One kit of Start Positive® Controls comprises:

- a pCMV-GFP plasmid complexed to the Viromer® reagent
- a GFP encoding mRNA complexed to the Viromer® reagent

Product information

Applications

Viromer® RED and **Viromer® YELLOW** are optimized for *in vitro* transfection of pDNA and mRNA.

Content and formats

Viromer® RED Incl. Buffer RED	100 transfections	VR-01LB-00
	900 transfections	VR-01LB-01
	3 x 900 transfections	VR-01LB-03
Viromer® YELLOW Incl. Buffer YELLOW	100 transfections	VY-01LB-00
	900 transfections	VY-01LB-01
	3 x 900 transfections	VY-01LB-03

In standard conditions (no optimization), **0.2µl of Viromer® is sufficient for 1 reaction of transfection in the 24-well plate format.** Standard size packs have 180µl of concentrated Viromer® and 50 ml of buffer.

Buffer RED and Buffer YELLOW (pH 7.2 aqueous solutions) are required for diluting the Viromer® reagents and pDNA or mRNA.

Storage and use

Viromer® RED and **Viromer® YELLOW** should be stored at **+2-8°C** in the provided aluminum bags. They are then stable for 6 months (#VR-01LB-00/#VY-01LB-00) to **1 year** (#VR-01LB-01/#VR-01LB-03/#VY-01LB-01/#VY-01LB-03). As the reagents are sensitive to atmospheric CO_2, it is recommended to always close the vials and tighten the caps immediately after use. Avoid contact of the reagents with dry ice.

Quality control

Each batch of Viromer® is tested for transfection using a luciferase reporter. Buffer solutions are analyzed for composition, sterility and RNase/DNase activity. MSDS are available at www.viromer-transfection.com.

Product use limitations

These products are intended **for research use only**; they must not be used for therapeutic, veterinary or diagnostic applications. The purchase of Viromer® reagents implies a limited, non-transferable right to the purchaser to use these products, or parts from these products, only for its internal research. All further commercial applications of Viromer® products require a license from Lipocalyx GmbH.

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VIROMER® **RED**

VIROMER® **YELLOW**

2017