Important recommendations

Viromer® YELLOW is fully compatible with cell culture media, sera or antibiotics. Seed cells in complete medium the day before transfection and replace with fresh medium before starting the experiment.

Cell culture and plating: Grow cells to reach 60...80 % confluency at the day of transfection. Use the volume of complete medium as mentioned in the table below.

Multiwell plate type	96	24	6
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

Suspension cells: These cells need more DNA, please start using the 1.5 x transfection scale and go to 2.0 x or 2.5 x. See our complete online manual for further recommendations.

Forward/reverse transfection: In forward transfection protocols, cells are seeded the day before transfection and the transfection complexes are freshly prepared at the transfection day. Instructions for reverse transfection and use in high-throughput screening (HTS) are provided in the detailed manual on the website.

For further optimization, information and trouble-shooting, go to **www.viromer-transfection.com** and visit our support pages.
...do not hesitate to contact us!

Version Dec / 15

VIROMER® YELLOW Quick Guide

pDNA/mRNA transfection

Detailed manual on Lipocalyx website
www.viromer-transfection.com

Important!

Avoid contact of Viromer® YELLOW with dry ice. Always close the vial and tighten the cap immediately after use. Do not vortex. Storage: +2-8°C within the provided aluminum bag.

Protocol steps:

- Day 0: Plating of cells
- Day 1: Transfection (preparation 10 min, incubation 15 min)
- Day 1-3: Final incubation before analysis (6-24h after transfection)

Conditions of use and required materials:

Warm all reagents to room temperature. Complexes should be prepared freshly. Use sterile, DNase/RNase free and apyrogenic tips and tubes.

Transfection Protocol: 3-condition optimization

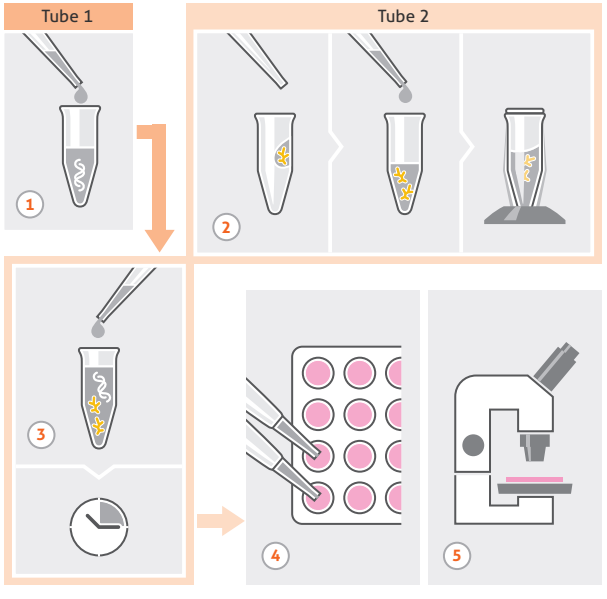
Volumes given here support 24 or 96 well formats. For 6 well, scale up 4 fold.

1. Dilute your pDNA/mRNA to 11 ng/µl using Buffer YELLOW. Provide a volume of 135 µl. ›› Tube 1
2. Place a 0.6 µl droplet of Viromer® YELLOW onto the wall of a fresh tube. Immediately add 14.4 µl of Buffer YELLOW and vortex for 3-5 s. ›› Tube 2
   Always add Buffer YELLOW to Viromer® YELLOW, not vice versa!
3. Pipette 135 µl of the pDNA/mRNA solution from Tube 1 onto the 15 µl of the Viromer® YELLOW solution in Tube 2. Mix swiftly and incubate for about 15 min at room temperature.
4. Add transfection complexes from step 3 to your cells. Titrate as per the table below to identify optimal conditions.

	96 well		24 well		6 well	
Transfection Scale	Transfer Volume per well	pDNA/ mRNA per well	Transfer Volume per well	pDNA/ mRNA per well	Transfer Volume per well	pDNA/ mRNA per well
low 0.5 x	5 µl	50 ng	25 µl	250 ng	100 µl	1000 ng
standard 1.0 x	10 µl	100 ng	50 µl	500 ng	200 µl	2000 ng
high 1.5 x	15 µl	150 ng	75 µl	750 ng	300 µl	3000 ng
	5 x replicates		1 x replicate		1 x replicate	

5. Incubate cells as usual. Monitor pDNA/mRNA effects 6-24 h after transfection. Expression from mRNA can begin as early as 2 h.

Workflow



Final Transfection Protocol

During optimization you identified a specific transfer volume and scale. Please proceed with your optimal settings.

The table below is a protocol using the 1.0 x transfection scale (standard). Please adjust all volumes according to your optimal transfection scale.

1. Start with diluting pDNA/mRNA to 11 ng/µl using Buffer YELLOW.

	96 well	24 well	6 well	comments
2 **Viromer® YELLOW**	0.6 µl	0.6 µl	2.4 µl	Buffer YELLOW onto Viromer® YELLOW Vortex immediately (3-5 s)
Buffer YELLOW	14.4 µl	14.4 µl	57.6 µl	
3 pDNA/mRNA in solution from Step 1	135 µl	135 µl	540 µl	Mix swiftly and incubate for 15 min
Viromer® YELLOW solution from Step 2	15 µl	15 µl	60 µl	
4 Transfer volume	10 µl	50 µl	200 µl	Incubate cells as usual
replicates	15 x	3 x	3 x	

5. Expression from pDNA/mRNA can be monitored from 6 to 24 h after transfection. Expression from mRNA can begin as early as 2 h.