VIROMER®ONE

standardized transfection reagent

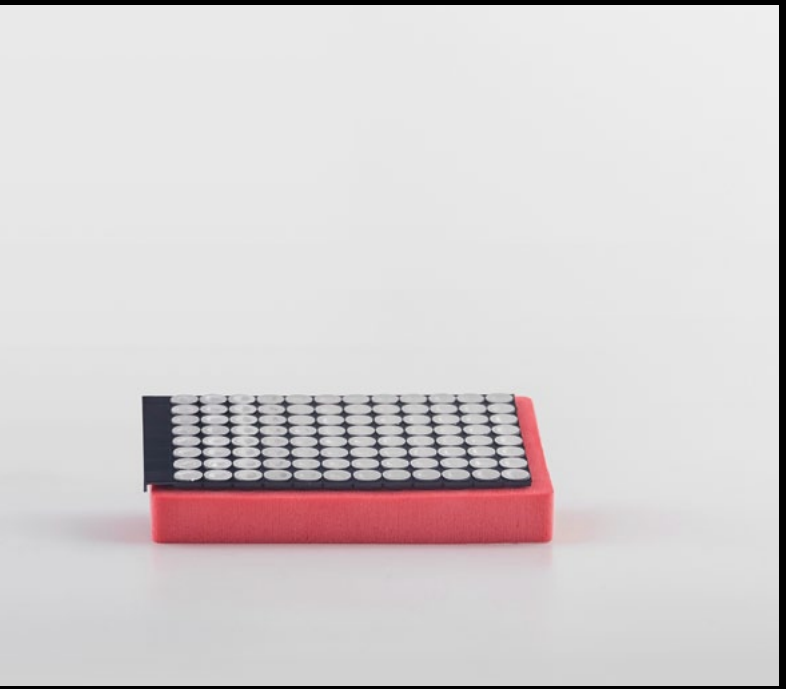
Doing transfection is like making coffee. Same procedure, somewhat different result each time you do it. Then came Nespresso. An easy, comfortable and clean way to make your prep.

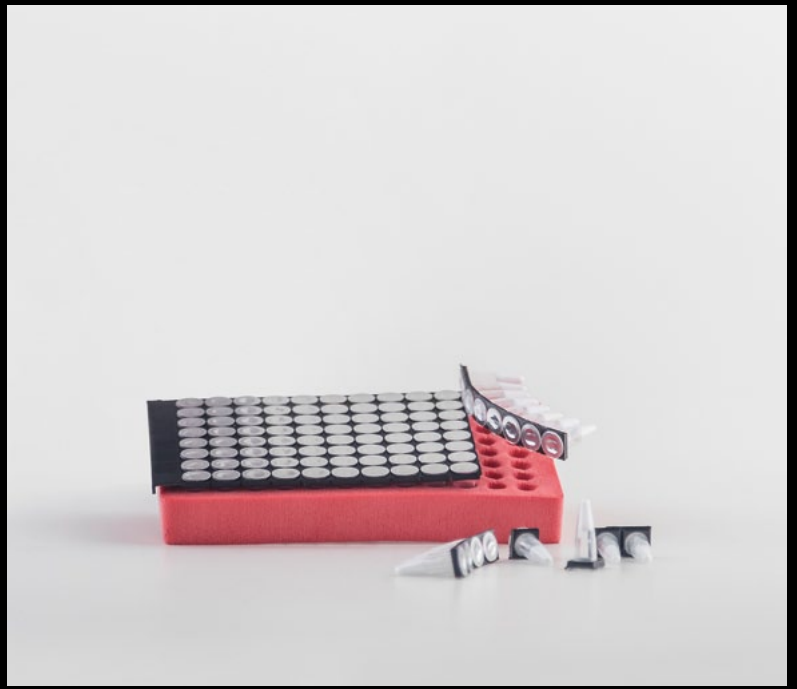
Regarding coffee we leave it to you. But for standardized transfections, there is **Viromer® ONE RED**. Start the tour and learn how simplicity and control became ONE.

VIROMER®ONE RED

Standardized, High-Performance Transfection

What's this?





Viromer® ONE RED is available as a full plate of 96 individual vials, each containing ONE portion of lyophilized ready-to-use transfection reagent. Simply break a tube or more. Add DNA or RNA. Get results. Store the rest for next time or share it with your labmates!

ONE step transfection

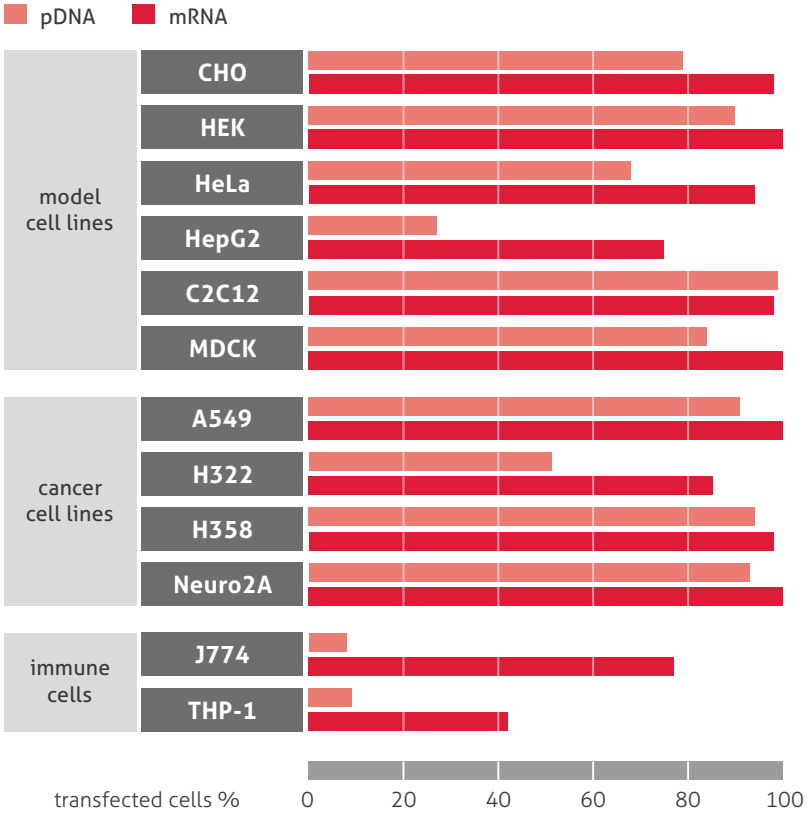
Designed for single to multichannel transfections in 24- to 96-well plate formats. Just add your diluted DNA (or mRNA) to suspend the reagent and enable complexation. ONE tube works for ONE well.

VIROMER® ONE RED

+ Standardized	> Preformed, calibrated reagent.
+ Clean	> Single portions keep the product fresh until use.
+ ONE step protocol	> Just add DNA! Or mRNA...
+ Easy-adjustable	> One round of a pre-set optimization.

Easy. Fast. Reliable.

Benchmark data:
Proven high-performance transfection



Transfection efficiency of Viromer® ONE RED used for plasmid DNA and mRNA delivery in a broad spectrum of cell lines.

Percentages of positive cells (max.) after transfection of a GFP-plasmid (3.5kb) or a GFP encoding mRNA (996nt).

- 50-150ng DNA or mRNA per 96-well depending on optimal rehydration and transfer volumes (for details, see page 11-12)
- read-out: 24 hours post-transfection by FACS Calibur

Viromer® ONE RED
achieves high-performance transfection of easy-to-transfect **model cell lines**. Its capacity to deliver mRNA even increases final protein expression and oversteps some of the limitations relative to plasmid transfection.

Viromer® ONE RED
shows comparable strong efficiency for transfecting plasmid DNA and mRNA into more specific cell types as **cancer cell lines**.

Viromer® ONE RED
in combination with mRNA is THE alternative to properly overexpress genes in "resistant" cells as **macrophages / monocytes.**

Basic Transfection Protocol

1 Preparation of pDNA / mRNA

- Dilute your pDNA/mRNA stock solution in water at **10 ng / µl**.
- Prepare a volume of **80 µl**.

2 Complexation

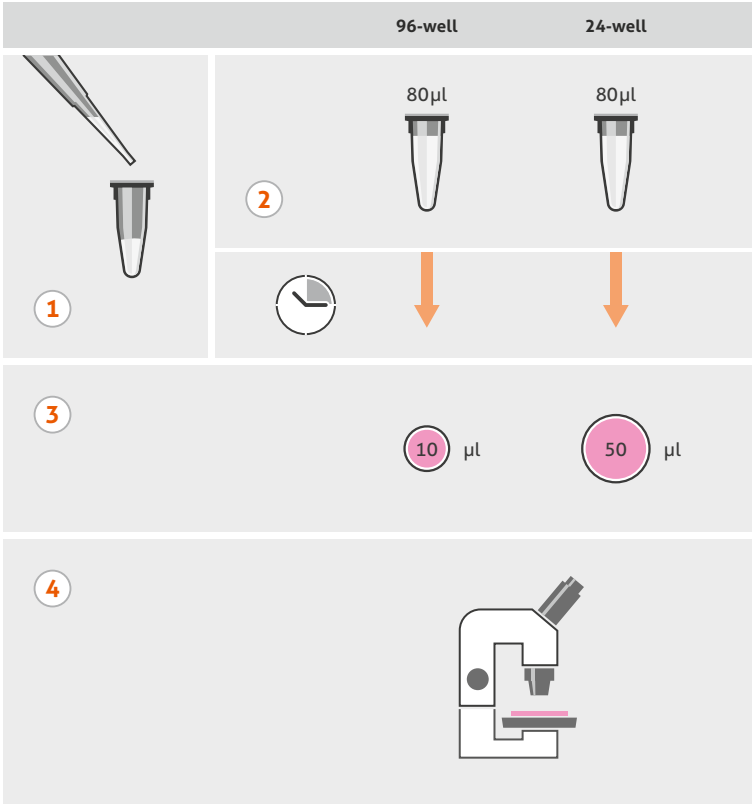
- Pierce the foil and rehydrate one vial with 80 µl.
- Mix swiftly by pipetting up and down and incubate for 15 min at room temperature.

3 Add the transfection complex on the cells

Per well	96-well	24-well
Transfer volume	10 µl	50 µl
pDNA / mRNA on cells	100 ng	500 ng

4 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.



Optimization Guide

The transfection efficiency is too low or there is toxicity?

Try this 96-well optimization scheme to vary

- **the Viromer®-pDNA (or –mRNA) ratio**
- **the amount of transfection complexes arriving onto the cells**

1 Preparation of pDNA / mRNA

- Dilute your pDNA/mRNA stock solution in water at **10ng/µl**.
- Prepare a volume of **400µl**.

2 Complexation

- Rehydrate 6 vials with volumes of 40-90µl.
- Mix swiftly by pipetting up and down and incubate for 15min at room temperature.

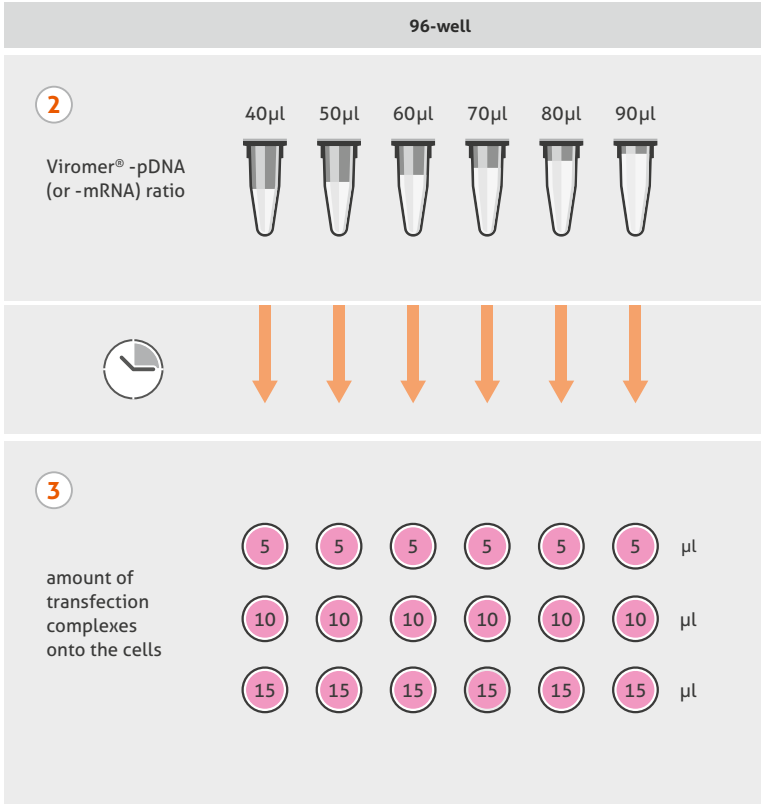
3 Add the transfection complex on the cells

Transfer complex onto the cells with 3 different volumes:
5, 10 and 15µl corresponding to 50, 100 and 150ng of DNA per well, respectively.

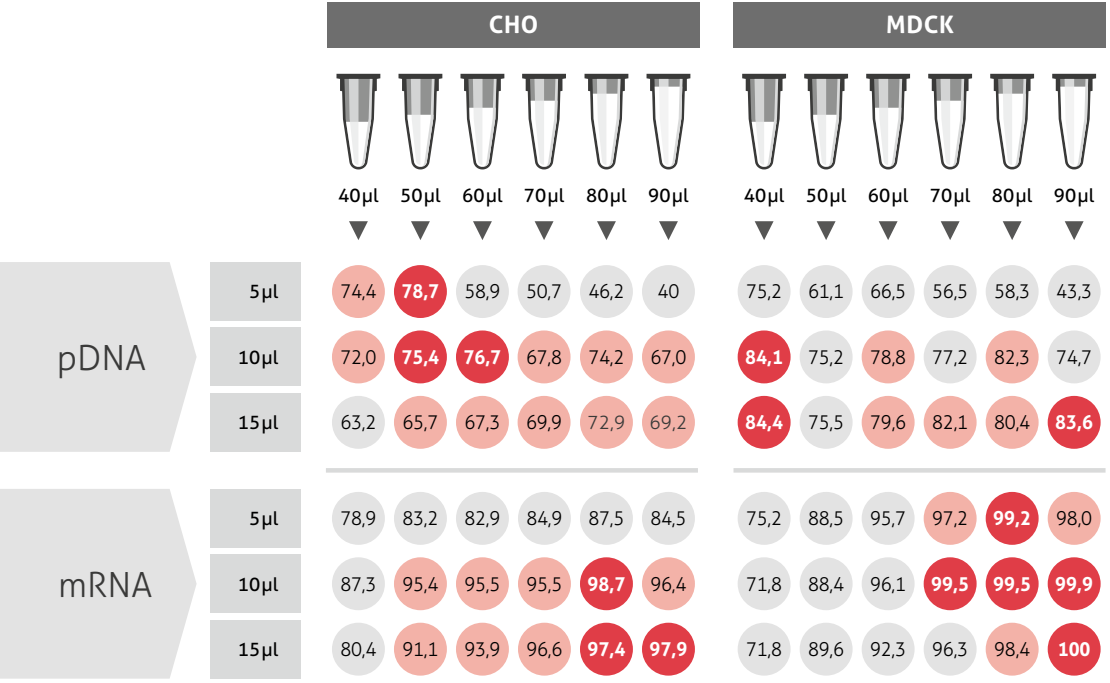
4 Read-out

Incubate cells and monitor effects as previously described.

Note: the **cell density** at seeding time (usually one day prior transfection) and the **duration of incubation** between transfection and monitoring of protein expression are also adjustable parameters. If unknown, the best conditions for your cells should be determined empirically beforehand.



Optimization - find the best conditions for your special cells and targets.



We applied the previously detailed optimization scheme to identify the best conditions to transfect 12 different cell lines.

Example of optimization data for pDNA and mRNA transfections in CHO and MDCK cells with Viromer® ONE RED.

Numbers in circles corresponds to the percentage of positive cells 24 hours post-transfection of a GFP plasmid (3.5kb) and a GFP encoding mRNA (996nt) depending on the rehydration volume of Viromer® ONE RED vials (40-90µl of diluted pDNA or mRNA at 10ng/µl) and the volume of transfection complex transferred onto plated cells (5, 10 or 15µl per 96-well, corresponding to 50, 100 and 150ng DNA or mRNA per well, respectively). Read-out: FACS Calibur.

Take a look at our in-house reference data.

		CHO	HEK	HELA	HepG2	C2C12	MDCK
	Cell density (cells/96-well)	1.0 x 10^4	2.2 x 10^4	15 x 10^5	3.5 x 10^4	7.0 - 10^3	9.0 x 10^3
pDNA	MAX % GFP+cells	79%	90%	68%	27%	99%	84%
	with Viromer® ONE RED rehydration	50µl	50-60µl	80µl	70µl	80-90µl	40µl
	transfer volume/well	5 µl	5 µl	10 µl	15 µl	15 µl	15 µl
	DNA/well	50 ng	50 ng	100 ng	150 ng	100-150 ng	150 ng
mRNA	MAX % GFP+cells	99%	100%	94%	75%	98%	100%
	with Viromer® ONE RED rehydration	80µl	80-90µl	80µl	90µl	90µl	90µl
	transfer volume/well	10 µl	15 µl	5 µl	10 µl	15 µl	15 µl
	RNA/well	100 ng	100-150 ng	50 ng	100 ng	150 ng	100-150 ng
		modell cell lines					

And try it on your own!

		A549	H322	H358	Neuro2A	J774	THP-1
	Cell density (cells/96-well)	5.0×10^3	2.5×10^4	2.0×10^4	1.2×10^4	2.0×10^4	6.0×10^4
pDNA	MAX % GFP+cells	91%	52%	94%	93%	8%	9%
	with Viromer® ONE RED rehydration	50µl	90µl	70µl	80µl	60µl	80µl
	transfer volume/well	5 µl	15 µl	15 µl	10 µl	15 µl	5 µl
	DNA/well	50 ng	150 ng	150 ng	100 ng	150 ng	50 ng
mRNA	MAX % GFP+cells	100%	85%	98%	100%	77%	42%
	with Viromer® ONE RED rehydration	80µl	90µl	60µl	50µl	80µl	90µl
	transfer volume/well	15 µl	15 µl	10 µl	15 µl	10 µl	10 µl
	DNA/well	50-150 ng	150 ng	100 ng	150 ng	100 ng	100 ng
		cancer cell lines				immune cells	

Why and how switching to mRNA transfection?

As shown in the previous set of data, 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® ONE RED has 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.

Use the Viromer® Start Positive® Controls!

Positive® Controls are pre-formulated **Viromer® ONE RED** 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 (1 vial)
- a GFP encoding mRNA complexed to the Viromer® reagent (1 vial)

Product information

Applications

Viromer® ONE RED is optimized for *in vitro* transfection of pDNA and mRNA.

Content and formats

Viromer® ONE RED	96 transfections	VR-01PF-01
Viromer® ONE RED +pDNA-/mRNA-GFP controls	96 transfections 150µl each	VR-PFBUNDLE-01

ONE plate is sufficient for 96 individual transfections in a 96-well or 24-well format. Control kits consist of lyophilized Viromer® RED already complexed with pCMV-GFP plasmid (1 vial) and GFP mRNA (1 vial).

Storage and use

Viromer® ONE RED should be stored at **+2-8°C** in the provided aluminum bag. The sealed package is stable for **6 months**. Use within 3 months when opened.

Quality control

Each batch of Viromer® is tested for transfection using a luciferase reporter. MSDS are available at www.viromer-transfection.com.

Product use limitations

This product is intended **for research use only**; it 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.

General information

Technology

Viromer® ONE RED is a lyophilized polymer-based transfection reagent featuring a viral mechanism of membrane fusion. It forms 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.

It is highly effective on a wide range of standard and hard-to-transfect cells including suspension cells, stem cells and primary 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, talk to our customer service or distributors.

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