THE VIROMER® FACTBOOK

Transfection

of siRNA, mRNA and plasmids

2017
Welcome to Viromer® Transfection Reagents! We provide:

- High transfection efficiency
- Great safety
- Easy and fast transfection with consistent results

Due to active escape of Viromer® complexes from the endosome

Because Viromer® complexes are non-charged, gentle on cells, and compatible with serum and antibiotics

Ascribed to a straightforward protocol, including initial optimization!

All features combined generate excellent results in challenging cells such as primary- and suspension cultures, macrophages and stem cells shown in this booklet. We thank our supporters for sharing their valuable data with us and the growing community of Viromer® users.

Want to learn about the Viromer® technology? Page 28 has the answers!
Product information, support, and our list of distributors to be found on pages 32 - 35

Transfection of siRNA and miRNA

Viromer® BLUE
Versatile for standard and challenging cells

Viromer® GREEN
Selected for specific cells such as THP-1, fibroblasts, and colon carcinoma

Viromer® RED
Versatile for standard and challenging cells

Viromer® YELLOW
Selected for specific cells such as primary cardiac myocytes, and hepatocytes
Viromer® BLUE – Very Efficacious Knockdown in Macrophages and Monocytes

RAW264.7: Mouse Macrophage-like Cell Line

Primary Human Macrophages

3774: Mouse Macrophages

Viromer® BLUE and GREEN – Powerful and Complementary Tools for Gene Silencing

Percentage of target mRNA

nm sirNA

EC50 = 0.6 nM

RAW 264.7

0,01 0,1 1 10 100

0

50

100

150

Viromer® bLuE and GREEN – Powerful and Complementary Tools for Gene Silencing

EC50 = 15nM

THP-1

0,01 0,1 1 10 100

0

50

100

150

Viromer® bLuE – Very Efficacious Knockdown in Macrophages and Monocytes

Primary Human Macrophages

THP-1 Monocytes

Primary Human M2-Macrophages

Examples of western-blots showing knock-down efficiency in macrophages and monocytes transfected with Viromer® bLuE.

Reduction of AHA-1 mRNA expression in RAW macrophages (upper) and THP-1 monocytes (bottom) by siRNA transfection with Viromer® BLUE (AHA-1 siRNA and control siRNA as filled and open symbols, respectively).

Acute

control siRNA

target siRNA

loading control (vinculin)

GaPDh

siRNA
cTS b

160414

cTS b si

160415

0

50

100

150

200

250

Viromer® BLUE and GREEN – Powerful and Complementary Tools for Gene Silencing

Primary Human Monocyte-derived Macrophages

Pink

control siRNA 1

target siRNA 2

control siRNA 1

target siRNA 2

control siRNA 1

target siRNA 2

control siRNA 1

target siRNA 2

Comparison of knock-down efficiencies with 2 different siRNA (100nM) in macrophages transfected by electroporation (NeoN®) or with the Viromer® technology (Western-blot and RT-qPCR after 96h).

Primary Human M2-Macrophages

Data from S. Barnard, University Hospital Halle, Germany

Data from A. Zhelankin, Bukrinsky’s lab, The George Washington University, USA

Examples of western-blots showing knock-down efficiency in macrophages and monocytes transfected with Viromer® BLUE.

Viromer® BLUE – Very Efficacious Knockdown in Macrophages and Monocytes

Examples of western-blots showing knock-down efficiency in macrophages and monocytes transfected with Viromer® BLUE.

THP-1 Monocytes

Primary Human M2-Macrophages

Primary Human M2-Macrophages

3774: Mouse Macrophages

Data from Dr. S. Medunjanin, University Hospital Magdeburg, Germany

Data from M. Klöse, University Hospital Hamburg, Germany

Experiment 1: Neutral Charge results in No Aggregation and High Performance in Suspension Cells

Data from J. Lung, Chang Gung Memorial Hospital, Chiayi, Taiwan

Data from Pelka, Univ Bonn, Germany (1,2); Ganthier, Hudelson Inst., Clayton, Australia (3); Schupp, University Mainz, Germany (4,5)

“Use Viromer® BLUE for transfection of PBMCs from buffy coats with a FITC labeled siRNA getting a very high transfection efficiency of 95% after 24h. I have used your product before with Lipofectamine which I had used previously.

M. Ballbach, University Tübingen, Germany

“The result was exciting and encouraging. The delivery efficiency is over 90% with good fluorescence intensity.”

J. Lung, Chang Gung Memorial Hospital, Chiayi, Taiwan

Microscopic observation of a labeled siRNA in KG-1a cells after transfection with Viromer® BLUE.

FGSc-h / FGSc-height

FSc-h / FSc-height

FL1-h / FL1-height

Data collected by Avallo, Kristbach, Germany

“Viromer® BLUE is the best transfection reagent.”

M. Ballbach, University Tübingen, Germany

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“Viromer® BLUE is the best transfection reagent.”

M. Ballbach, University Tübingen, Germany

Microscopic observation of a labeled siRNA in KG-1a cells after transfection with Viromer® BLUE.
Macrophages can take up DNA... but mRNA is a much better choice...

Expression of proteins from DNA in macrophages is limited to certain cell lines like RAW 264.7 and is not always reliable. Primary macrophages or THP-1 monocytes cannot process DNA as part of the innate immune system. Once imported into the cytosol, AIM-2 and cGAS recognize double-stranded DNA before it can reach the nucleus and inhibit further processing.

Use of mRNA is the solution to this problem. Being a natural component of the cytosol, mRNA provides a very productive template for translation.

Viromer® RED and Viromer® YELLOW transfection products have been optimized to work equally strong with DNA and mRNA. Our Start Positive® Controls easily guide you on the transition to the RNA world to maximize your results.

Viromer® RED transfects 90% of Human Primary Monocytes with mRNA... and yields bright results in immune cells!

Viromer® RED transfects 90% of Human Primary Monocytes with mRNA.

Transfection of primary human macrophages with pcMV-GFP plasmid and GFP-encoding mRNA using Viromer® RED Start Positive® Control. Expression of GFP was only observed with mRNA, no signal with plasmid DNA. Contributor not disclosed.
Viromer® BLUE and GREEN – Powerful Tools for Cancer Research

CT26: Colorectal Carcinoma

Western Blot shows total reduction of Stat3 protein using its si rNa complexed to Viromer® GREEN.

Scrambled control si rNa has no effect on Stat3 protein levels.

Hs746T cells were transfected with GAPDH si rNa (25nM) for 72h.

Viromer® BLUE efficiently and safely transfected Hs746T cells. Expression of GAPDH mRNA was reduced by 90% without any signs of toxicity.  
Gartner, Novartis, USA

H295R: Adrenocortical Carcinoma Cells

Knock-down efficiency in H295R adrenocortical carcinoma cells after transfection with Viromer® GREEN and Viromer® BLUE.

Data from Dr. S. Sbiera, University Hospital Würzburg, Germany

P4E6: Human Prostate Carcinoma Cells

Comparative knock-down efficiency of Viromer® BLUE with 3 other commercial reagents.

Microscopic observations of P4E6 cells transfected with a red-labeled si rNa (20nM) by using Viromer® BLUE.

Knock-down efficiency in H295R adrenocortical carcinoma cells after transfection with Viromer® BLUE.

Viromer® BLUE and GREEN – Powerful Tools for Cancer Research

Primary human and LS174T Colon Carcinoma Cells

Comparative knock-down efficiency of Viromer® BLUE with 3 other commercial reagents.

Microscopic observations of P4E6 cells transfected with a red-labeled si rNa (20nM) by using Viromer® BLUE.

Contributor not disclosed

Comparative knock-down efficiency of a self-established melanoma cell line after transfection of control and 2 target si rNa with Viromer® BLUE and Lipofectamine® 2000.

Contributor not disclosed

Comparison of knock-down efficiency in a self-established melanoma cell line after transfection of control and 2 target si rNa with Viromer® BLUE and Lipofectamine® 2000.

Contributor not disclosed
Viromer® RED outperforms Major Competitors on Hard-to-Transfect Cancer Cells

**C6: Glioma Cells**

Viromer® RED

FuGene® hD

TransIT-X2

**SH-SY5Y: Neuroblastoma Cells**

Viromer® RED

FuGene® hD

Lipofectamine® 2000

**MCF-7 Cells**

Viromer® RED

FuGene® hD

Lipofectamine® LTX

*Given the efficiency we saw, your product might be uniquely poised to address many research questions using this clinically relevant cell line. Viromer Red showed faster and better transfection efficiency in Glioma cell line as compared to Lipofectamin.”

© 2016 Pearse Lab, Miami Project to Cure Paralysis, Dr. S. Rao, USA

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**Viromer® RED and YELLOW – Effective Protein Expression in Challenging Cancer Cells**

Ntera-2 Cells

GFP expression in Ntera-2 cells transfected with Viromer® RED (50% of transfected cells, no toxicity).

Data from Dr. L. Marcos-Villar, Dr. A. Nieto’s group, Centro Nacional de Biotecnología / CNB-CSIC, Spain

**Mouse Erythroleukemia (MEL) Cells**

GFP expression in MEL cells transfected with Viromer® RED (50% of transfected cells, no toxicity).

Data from N. Wright, Sheffield Hallam University, UK

**MCF-7 Cells**

Luciferase Activity (RLU)

Viromer® RED

FuGene® hD

Lipofectamine® 2000

Cell viability [%]

FuGene® hD

Lipofectamine® LTX

Viromer® RED

Lipofectamine® 2000

Lipofectamine® LTX

Expression of GFP in MCF-7 cells transfected with Viromer® RED.

Data generated by Lipocalyx, Halle, Germany.

Viromer® RED outperforms Major Competitors on Hard-to-Transfect Cancer Cells

**SH-SY5Y: Neuroblastoma Cells**

Increasing number of transfected cells from 24h to 72h (pictures taken at 72h)

Viromer® RED

FuGene® hD

TransIT-X2

**MCF-7 Cells**

Viromer® RED

FuGene® hD

TransIT-X2

**SH-SY5Y: Neuroblastoma Cells**

Viromer® RED

FuGene® hD

TransIT-X2

**MCF-7 Cells**

Viromer® RED

FuGene® hD

TransIT-X2

**Mouse Erythroleukemia (MEL) Cells**

GFP expression in MEL cells transfected with Viromer® RED (50% of transfected cells, no toxicity).

Data from N. Wright, Sheffield Hallam University, UK

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**AS49 Lung Adenocarcinoma Cells**

Expression of GFP in AS49 cells transfected with Viromer® RED (after 24h, approx. 80% of transfected cells, no toxicity).

Data from Dr. L. Marcos-Villar, Dr. A. Nieto’s group, Centro Nacional de Biotecnología / CNB-CSIC, Spain

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Given the efficiency we saw, your product might be uniquely poised to address many research questions using this clinically relevant cell line. Viromer Red showed faster and better transfection efficiency in Glioma cell line as compared to Lipofectamin.”

© 2016 Pearse Lab, Miami Project to Cure Paralysis, Dr. S. Rao, USA
Viromer® BLUE – Very Effective Knock-Down in Metabolic Cells

Viromer® BLUE and GREEN – Strong Knock-Down in Metabolic Cells

Knock-down of 2 proteins in primary mouse hepatocytes after transfection of 3 different siRNA with Viromer® BLUE.

Data generated by N.V. Calo, University of Geneva (Switzerland)
Hepatocyte Cell Line FAO

Expression of a glutamate membrane transporter tagged with GFP in COS-7 cells transfected with Viromer® RED. (Cell nuclei stained in red, approx. 80% of transfected cells)
Data generated by Prof. C. Perego, University of Milan, Italy

HepaRG™: Hepatic Stem Cells

Expression of GFP in HepaRG™ cells transfected in suspension with Viromer® RED (50ng DNA).
Contributor not disclosed

Primary Keratinocytes

Expression of GFP in primary keratinocytes transfected with Viromer® RED.
Data generated by M. Podgórska; Prof. S. Smola: University Hospital Homburg/Saar, Germany

COS-7 Fibroblasts

Expression of GFP in rat hepatocyte cell line FAO transfected with Viromer® YELLOW.

Viromer® RED outperforms Major Competitors

Comparison of luciferase activity and cell viability 24h post-transfection of mRNA (2kb) with Viromer® RED.
Data generated by Lipocalyx, Halle, Germany.
Viromer® BLUE and GREEN – Strong Knock-Down in Muscle Cells

**Primary Human Skeletal Myoblasts**

Knock-down of protein X in C2C12 myoblasts cells transfected with Viromer® BLUE.

Data generated by J. A. Zagalak, ETH Zürich – IPW, Switzerland

**Primary Human Myoblasts**

Knock-down of FGFR4 protein in RMS cells (22uM siRNA with Viromer® BLUE and Viromer® GREEN).

Data from Dr. M. Wachtel, University Children’s Hospital, Zürich, Switzerland

Viromer® BLUE – Efficient Knock-Down in Cardiovascular Cells

**RMS: Human Rhabdomyosarcoma Cells**

Knock-down of FGFR4 protein in RMS cells (22uM siRNA with Viromer® BLUE and Viromer® GREEN).

Data from Dr. M. Wachtel, University Children’s Hospital, Zürich, Switzerland

**C2C12 Myoblasts**

Knock-down of protein X in C2C12 myoblasts cells transfected with Viromer® BLUE.

Data generated by J. A. Zagalak, ETH Zürich – IPW, Switzerland

**H9C2 Cardiomyocytes**

Knock-down of IRAK 1 in H9C2 rat cardiomyocytes transfected with Viromer® BLUE (6h and 72h after transfection).

Data from Dr. M. Wachtel, University Children’s Hospital, Zürich, Switzerland

**Primary Human Myoblasts**

Knock-down of protein X in primary human myoblasts after transfection with a Cy3-labeled siRNA using Viromer® BLUE.

Data generated by J. A. Zagalak, ETH Zürich – IPW, Switzerland

**Microscopy observation of a labeled miRNA in H9c2 rat cardiomyocytes transfected with Viromer® BLUE.**

Data from J. Oh, Icahn School of Medicine at Mount Sinai, New York, USA

"Minor toxicity was observed but this was likely due to knockdown of the gene rather than toxicity from Viromer® itself." Contributor not disclosed

**Primary Human Skeletal Myoblasts**

Microscopy observation of human primary myoblasts after transfection with a Cy3-labeled siRNA using Viromer® BLUE.

Data generated by C. Weigert, University of Tübingen

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Microscopic observation of human primary myoblasts after transfection with a Cy3-labeled siRNA using Viromer® BLUE.

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Data generated by C. Weigert, University of Tübingen

"Minor toxicity was observed but this was likely due to knockdown of the gene rather than toxicity from Viromer® itself." Contributor not disclosed
Transfection of a pCMV-GFP plasmid and GFP encoding mRNA into C2C12 and HUVECs with Viromer® RED.

“I was very happy with the results. So far I had good results with the standard protocol and lowest concentration of Viromer Yellow. I have tried incubating the myocytes with the Yellow reagent for 4 h as suggested and the results were very good.”

N. Kaludercic, University of Padova, Italy

“Your transfection product (Viromer YELLOW) works much better than the other reagents we have used before to transfect neonatal rat cardiomyocytes. We obtain 50% efficiency with low toxicity.”

A. Castellano, University of Seville, Spain

Viromer® RED – Excellent Transfection in Muscle and Endothelial Cells

Viromer® RED and YELLOW – Outperform Standard Transfectants in Cardiomyocytes

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

Data generated V. Jayarajan, Charité Berlin, Center for Cardiovascular Research, Germany

Data from M. Nowoczyn, University Hospital Caen, France

The same experiment was repeated several times. Overall, with the ERE-CMV-EGFP plasmid, around 40-50% of the myoblasts showed green fluorescence with Viromer® RED. The efficacy of jetPRiME® was variable and usually quite low. Only in one experiment jetPRiME® was almost as efficient as Viromer® RED. Based on these findings, we selected Viromer® RED as the transfection reagent for further experiments with C2C12 myoblasts...

Viromer® RED and YELLOW – Outperform Standard Transfectants in Cardiomyocytes

“Your transfection product (Viromer YELLOW) works much better than the other reagents we have used before to transfect neonatal rat cardiomyocytes. We obtain 50% efficiency with low toxicity.”

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Viromer® RED – Excellent Transfection in Muscle and Endothelial Cells

Viromer® RED and YELLOW – Outperform Standard Transfectants in Cardiomyocytes
Viromer® BLUE and GREEN – Strong Knockdown in Nerve cells

Primary Microglia Cells

Glioblastoma Cells

Microscopic observation of siGLO-red transfected with Viromer® BLUE into primary microglia cells.

Transfection efficiency (%), light blue bars; and cell death (%), dark blue bars of glioblastoma cell lines after transfection of siRNA (at 10nM) using Viromer® BLUE.

The results are more than satisfying, considering that we were not able to transfect these cell lines with any other transfection reagent that is on the market. — M. Maleszewska, Nencki Institute of Experimental Biology Warsaw, Poland

Data from V. Mathur, T. Wyss-Coray’s Lab, Stanford University, Palo Alto, USA

50% Knock-down efficiency with Viromer® BLUE.

Contributor not disclosed

Cell lines & siRNA [nM] Target Gene knock-down efficiency % Data from
NCH82 10 – >80% Viromer® BLUE I. Dokic, DKFZ Heidelberg (Germany)
NCH149 10 – >90% Viromer® BLUE I. Dokic, DKFZ Heidelberg (Germany)
LN 229 10 EGF >70% Viromer® BLUE >90% Viromer® GREEN H. Wichmann, University Hospital Halle (Germany)
U-251 MG 10 EGF >75% Viromer® BLUE H. Wichmann, University Hospital Halle (Germany)
U-87 MG 10 HTRT >90% Viromer® BLUE IDT, Coralville (USA)

Viromer® BLUE and GREEN – Very Effective Knockdown in Nerve cells

Primary Mouse Astrocytes

Microscopic observation of a red-labeled miRNA mimic transfected in primary astrocytic cultures, isolated from postnatal mice p5, with Viromer® bLuE and Lipo-Fectamine® 2000 - Red: miRNA-Dy567 / Blue: TO-PRO-3.

E. Papadimitriou, D. Thomaidou, Department of Neurobiology - Hellenic Pasteur Institute, Greece

Transfection efficiency in pheochromocytoma cells transfected with Viromer® bLuE and Viromer® GREEN.

“We have a really poor transfection efficiency. We consider the results with BLUE and GREEN encouraging.” — Contributor not disclosed

Data from Pellagata’s Lab - Helmholtz Center Munich, Germany

Primary Mouse Brain Schwann Cells

MPC: Mouse Primary Pheochromocytoma Cells

Western Blot

A - Knock-down efficiency in BV-2 cells transfected using Viromer® BLUE.

B - Corresponding cell viability.

Data from V. Mathur, T. Wyss-Coray’s Lab, Stanford University, Palo Alto, USA

No sirNa

Viromer® bLuE

Viromer® GREEN

α-tubulin

Data from I. Pediaditakis & I. Charalampopoulos, University of Crete (Greece)
**Viromer® RED** – Non-toxic and Achievable Transfection in Neurons and Glial Cells

- **Primary Mouse Neurons**
- **DI TNC1 Rat Brain Astrocytes**
- **BV-2: Mouse Microglia-Like Cells**

Transfection of neurons with Viromer® RED opens great perspectives for challenging cells which currently require specific preparations and are mainly only accessible by electroporation or viral transduction.

### Microscopic Observations
- **Primary Mouse Neurons**
- **DI TNC1 Rat Brain Astrocytes**
- **BV-2: Mouse Microglia-Like Cells**

Data generated in collaboration with the Fraunhofer Institute for Cell Therapy and Immunology, Halle, Germany.

### Comparison of Transfection Efficiency

Viromer® RED and Lipofectamine® reagents in diverse murine glial cells.

**Primary Rat Oligodendrocytes**

Transfection of neurons with Viromer® RED opens great perspectives for challenging cells which currently require specific preparations and are mainly only accessible by electroporation or viral transduction.

### Microscopic Observations
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Data from S. Küspert, University of Erlangen, Germany.

### Microscopic Observations
- **SH-SYSY Neuroblastoma**
- **Primary Rat Oligodendrocytes**

Data from S. Rao - University of Miami, Miller School of Medicine, USA.

Data from F. Letournel, CHU Angers, France.

**Viromer® RED** – Efficient in Cancer Cell Lines and Primary Cells

Microscopic observations of SH-SYSY neuroblastoma transfected with pEGFP-N2 and pNFH-GFP by using Viromer® RED.

Data from F. Letournel, CHU Angers, France.

Microscopic observations of oligodendrocytes transfected with pMAX-GFP after 5 days of differentiation (in vitro) by using Viromer® RED.

Data from S. Küspert, University of Erlangen, Germany.
Transfection using Start Positive® Controls

Start Positive® controls are ready-to-use preformulated complexes for transfection of new cell types or as reference material. Start Positive® controls for Viromer® RED/YELLOW comprise of one pDNA and one mRNA sample each, facilitating comparative studies between these genetic cargoes.

Data generated by 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, Vaxcel 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.

Start Positive® Controls – Strong Transfection of Challenging Cells with plasmid DNA and mRNA

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Viromer® RED pDNA/mRNA Controls</th>
<th>Viromer® BLUE siRNA controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH-SYSY</td>
<td>pCMV-GFP (3.5kb) plasmid complexed to Viromer® RED</td>
<td>GAPDH-siRNA complexed to Viromer® BLUE</td>
</tr>
<tr>
<td>RAW 264.7</td>
<td>GFP mRNA complexed to Viromer® RED</td>
<td>non-targeted siRNA labeled with Cy3 complexed to Viromer® BLUE</td>
</tr>
<tr>
<td>MDSCs</td>
<td>pCMV-GFP (3.5kb) plasmid complexed to Viromer® YELLOW</td>
<td></td>
</tr>
<tr>
<td>Dendritic cells (MDDCs)</td>
<td>GFP mRNA complexed to Viromer® YELLOW</td>
<td></td>
</tr>
</tbody>
</table>

Data from F. Gueugnon, Vaxcel Research, CEA-Saclay, France
Viromer® Technology translates your Research to IN VIVO Applications

A lyophilized powder containing Viromer® nanoparticles. It only needs to be rehydrated with the diluted target mRNA to form active particles.

Viromer® IN VIVO mRNA transfection reagent facilitates effective systemic delivery and protein expression in animals. Systemic injection into the tail vein of a mouse results in rapid protein expression in liver and spleen. For luciferase, substantial activity as early as two hours upon injection was detected and the signal could be tracked for up to 44 hours.

Dose-dependent mRNA delivery in Mouse Liver and Spleen

Use of mRNA in combination with the Viromer® delivery system has become a versatile tool to achieve rapid and predictable gene expression in vivo.

Current modified mRNA chemistries have solved much of the instability associated with its native form as well as its capacity to elicit innate immune responses.

Viromer® IN Vivo has generated results upon systemic and local injection routes in spleen, liver, dermis, muscles, duodenum and peritoneum in laboratory mouse models.

Delivery can be tailored to a target organ. Mice received a single dose of luciferase encoding mRNA: Viromer® complex through a tail intravenous injection. Spleen and liver can selectively be addressed using low or high doses (10 to 40 µg) of material, respectively. (ventral view)

Transient mRNA expression. Time course of the mRNA signal after a single 10-µg tail intravenous injection. Luciferase expression can be detected into the spleen as early as 2h after injection (dorsal view).

Viromer® Technology translates your Research to IN VIVO Applications

VIROMER® IN VIVO mRNA Systemic Kit

- 6 vials of lyophilized Viromer® (30 rxns)
- 2 vials of lyophilized Positive Controls (2 rxns, Luc encoding mRNA complexed to Viromer®)

Technology translates your Research to IN VIVO Applications

A lyophilized powder containing Viromer® nanoparticles. It only needs to be rehydrated with the diluted target mRNA to form active particles.

Viromer® IN VIVO mRNA transfection reagent facilitates effective systemic delivery and protein expression in animals. Systemic injection into the tail vein of a mouse results in rapid protein expression in liver and spleen. For luciferase, substantial activity as early as two hours upon injection was detected and the signal could be tracked for up to 44 hours.

Dose-dependent mRNA delivery in Mouse Liver and Spleen

Use of mRNA in combination with the Viromer® delivery system has become a versatile tool to achieve rapid and predictable gene expression in vivo.

Current modified mRNA chemistries have solved much of the instability associated with its native form as well as its capacity to elicit innate immune responses.

Viromer® IN Vivo has generated results upon systemic and local injection routes in spleen, liver, dermis, muscles, duodenum and peritoneum in laboratory mouse models.

Delivery can be tailored to a target organ. Mice received a single dose of luciferase encoding mRNA: Viromer® complex through a tail intravenous injection. Spleen and liver can selectively be addressed using low or high doses (10 to 40 µg) of material, respectively. (ventral view)

Transient mRNA expression. Time course of the mRNA signal after a single 10-µg tail intravenous injection. Luciferase expression can be detected into the spleen as early as 2h after injection (dorsal view).
Viromer®: A novel polymer based transfection reagent mimicking the viral infection process by an active endosome escape mechanism.

In Influenza, the pH-sensitive fusion peptide inserts into the endosomal membrane. Mechanism relies on protonation of GLU, balanced by hydrophobic ALA.

Viromers mimic the Influenza mechanism, but use a polymer instead of a fusion peptide. Fatty acids (red) resemble Influenza’s GLU, alkyl (grey) are similar to ALA.

As a result, both influenza and Viromer promote an active endosome escape leading to cytosolic delivery.

Uncharged (grey) and charged (blue) groups regulate membrane transfer.

Viromers are taken up at the cell surface.

Viromers accumulate near the nucleus, ongoing acidification.

Discharge of siRNA from endosomes and starting diffusion.

Model: HeLa, Viromer® GREEN, labelled siRNA. Data courtesy of Chromotek.
Viromer® – Neutral Charge of Transfection Complexes are proven safe and effective in Suspension or 3D Cell Cultures

Viromer® is proven superior to Lipofectamine with 3D spheroids and show advanced performance in THP-1, PBMCs, or primary microglia cells. (See pages 4, 5 and 9)

“And thank you very much for letting us test the Viromer system, we were satisfied with the efficiency of knockdown.”

J. Holland, MDC Berlin

Viromer® – Safe Transfection with a Wide Working Range

The outcome of RNAi experiments depend on the ratio between the siRNA signal and any unspecific noise. Viromer® achieve signal: noise ratios between 20 and 30, much better than six leading competitors.

Very low background in a phenotypic assay. Cell cycle of HeLa was arrested using PLK-1 siRNA (filled symbols, the signal) or a control siRNA (open symbols, the noise). For Viromer® BLUE, the EC50 values differ by a factor of 30.

Signal: noise ratio for various transfectants. Signal: noise is defined as the ratio of EC50 values for the active and control siRNA.

Signal: noise ratios of transfectants

Viromer® BLUE phenotypic assay

Knockdown of target and GAPDH control in cancer mammospheres.

Neutral Charge

3D Culture of Cancer Stem Cells (Mammospheres)
## Products & Ordering

### Viromer® for siRNA/miRNA applications

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### Viromer® IN VIVO Product

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### Viromer® for plasmid/mRNA applications

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</table>

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### Online Ordering: [www.viromer-transfection.com](http://www.viromer-transfection.com)

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