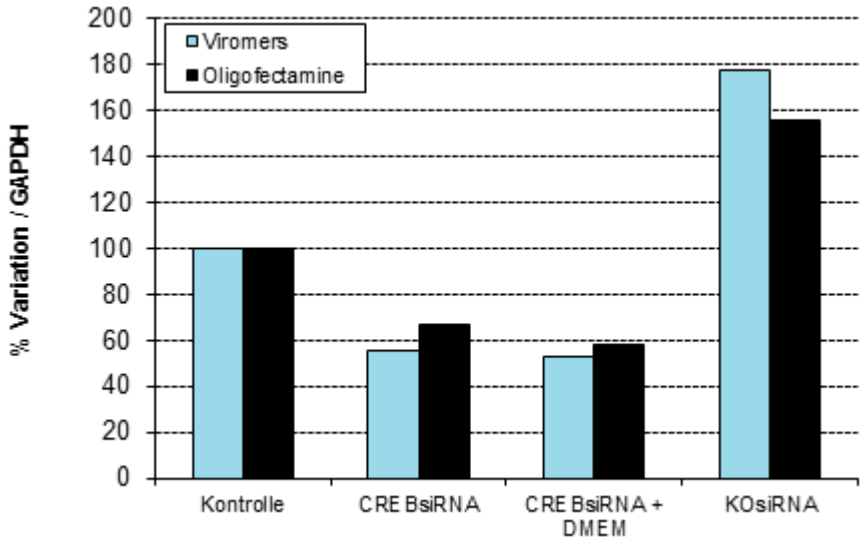
A549: transfection of siRNA



VB-01LB-00, VB-01LB-01

	CREB	GAPDH	Ratio	%	Produkt	Medium
1. Kontrolle **= only Viromer**	273,10	254,90	1,07	100,0	Viromer	Optimem
2. CREBsiRNA **= target siRNA**	171,92	287,24	0,60	55,9	Viromer	Optimem
3. KOsiRNA **= control siRNA**	517,44	271,29	1,91	178,0	Viromer	Optimem
4. Kontrolle **= only Oligofect**	249,16	248,02	1,00	100,0	Oligofectamine	Optimem
5. CREBsiRNA **= target siRNA**	195,81	291,93	0,67	66,8	Oligofectamine	Optimem
6. KOsiRNA **= control siRNA**	396,72	253,13	1,57	156,0	Oligofectamine	Optimem
7. CREBsiRNA + DMEM **= target siRNA**	136,00	238,25	0,57	53,3	Viromer	DMEM
8. CREBsiRNA + DMEM **= control siRNA**	158,63	271,13	0,59	58,2	Oligofectamine	DMEM

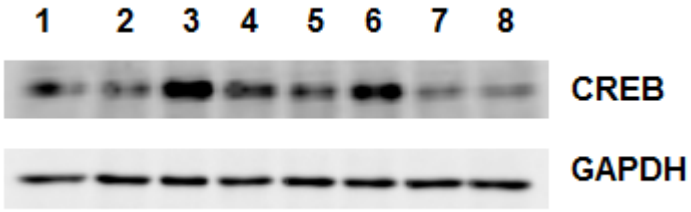


Fig. 1: Knock-down of CREB gene expression in A549 lung adeno-carcinoma cells transfected with Viromer® BLUE or Oligofectamine®

- Standard protocol (no optimization): 25nM siRNA
- Test with Optimem and DMEM + 5% FCS
- 4h post-transfection: change of medium for DMEM
- Read-out: 72h post-transfection (protein expression by WB)

>> Viromer gives the best answer (**47% knock-down**) with use of DMEM

Data from S. Wodischek, Dpt of Heart Surgery, Medicine University MLU-Halle (Germany)

A549: transfection of plasmid DNA

VR-01LB-00, VR-01LB-01

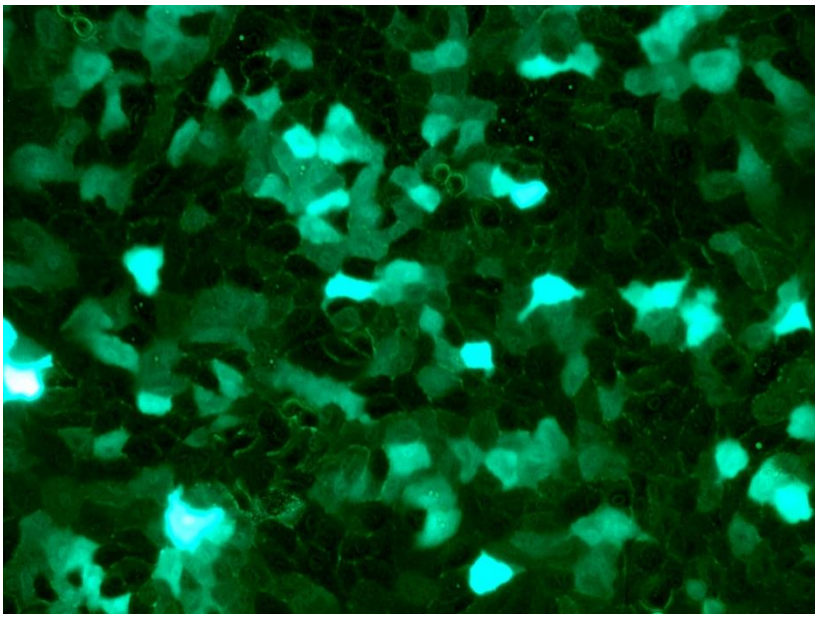


Fig. 2: Microscopic observation of GFP expression in A549 lung adenocarcinoma cells transfected with Viromer® RED

- standard protocol
- approx. **80% efficiency,** 24h post transfection
- good cell viability

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

A549: transfection of plasmid DNA

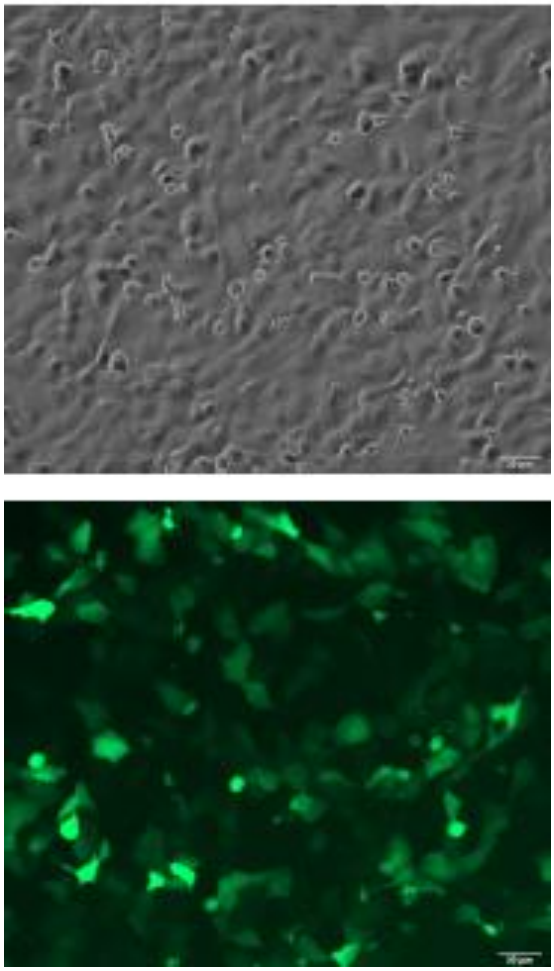
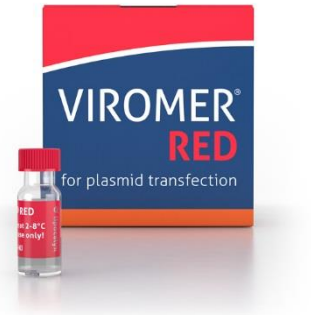


Fig. 3: Microscopic observation of GFP expression in A549 lung adenocarcinoma cells transfected with Viromer® RED



VR-01LB-00, VR-01LB-01

- **47% efficiency** 24h post transfection
- good cell viability

Data from Prof. Giehl, University of Gießen (Germany)

A549: transfection of plasmid DNA

VR-01LB-00, VR-01LB-01

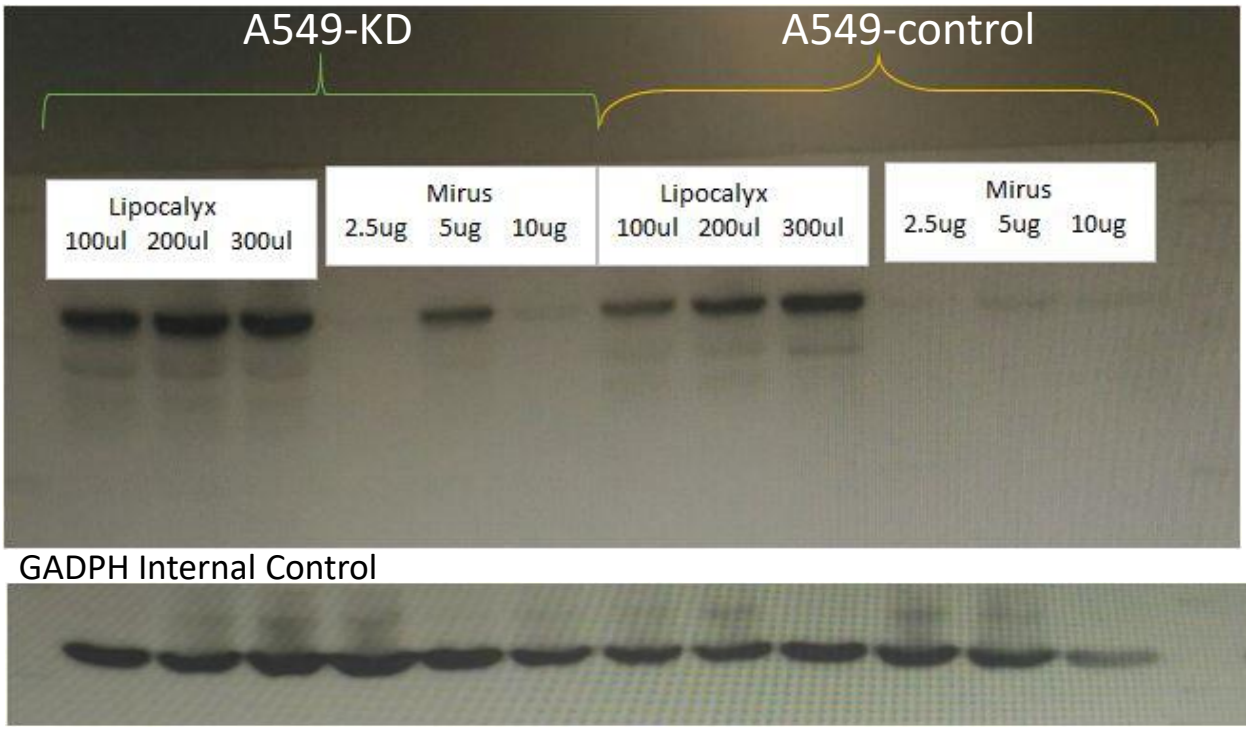


Fig. 4: Comparative protein expression in A549 lung adenocarcinoma cells transfected with MIRUS® reagent and Viromer® RED at 3 different transfection scales

- 6-well plate format
- Final DNA amount per well: 1, 2 and 3 µg
- Viromer>>Mirus: efficiency, cell viability, convenience of use

Data from Prof. LM Huang, Dpt of Pediatrics, National Taiwan University Hospital (Taiwan)

A549: transfection of plasmid DNA

VR-01PF-01

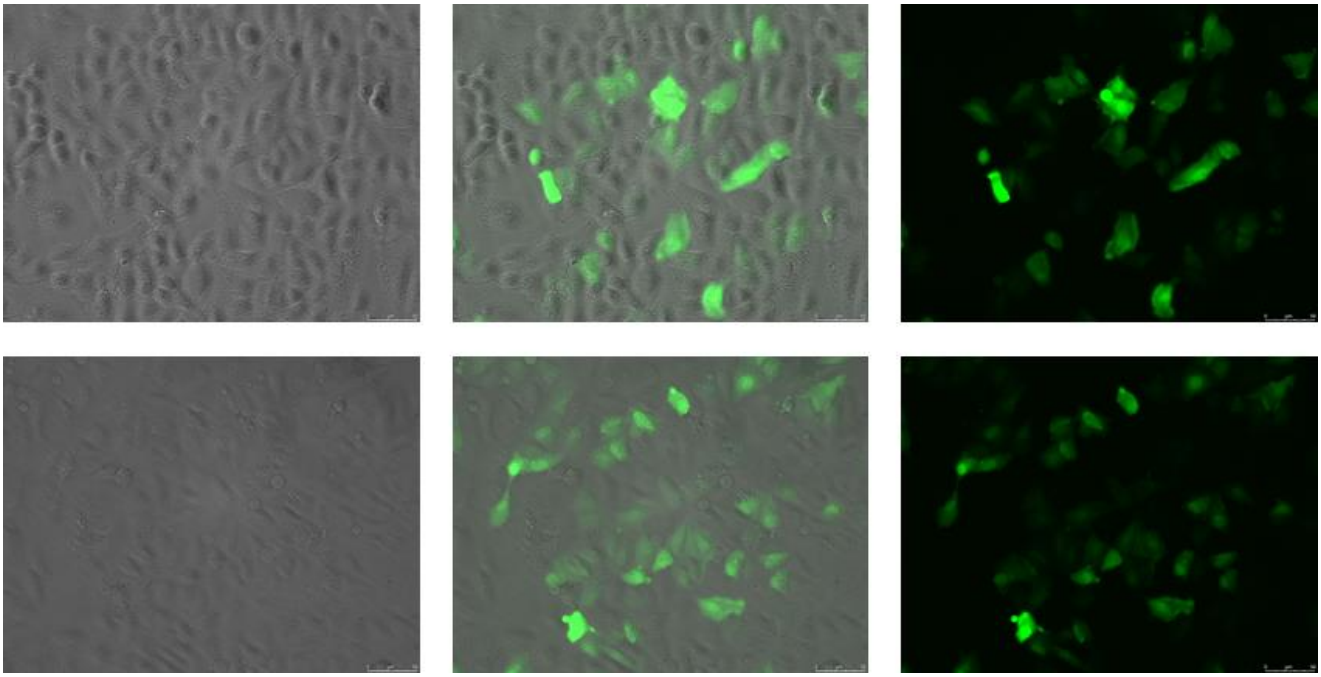
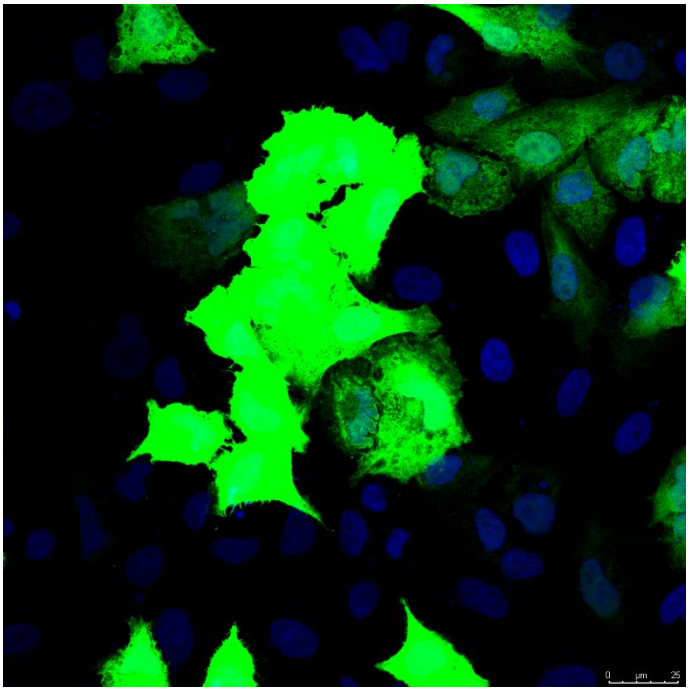


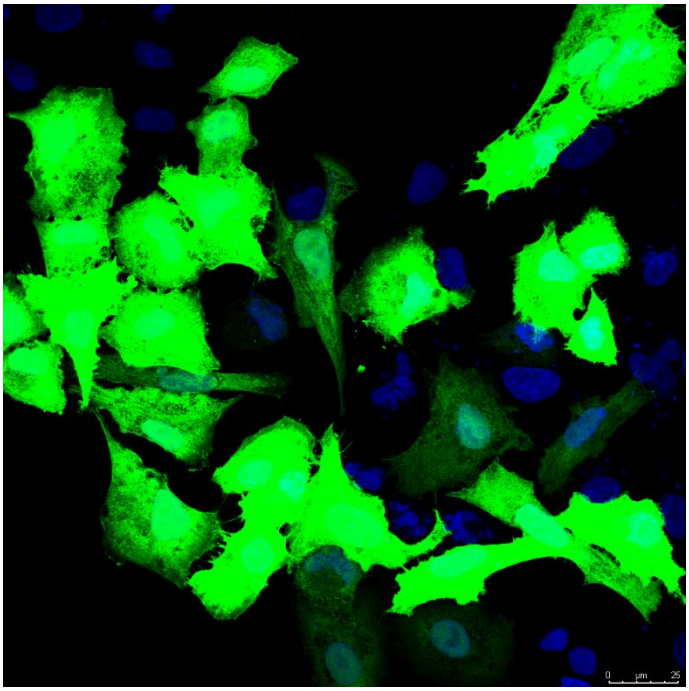
Fig. 5: Microscopic observations of GFP expression in A549 lung adenocarcinoma cells transfected with Viromer® ONE RED

- standard protocol, cells seeded at 75% confluency
- approx. **70% efficiency,** 24h post transfection
- good cell viability

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

A549: transfection of plasmid DNA





VR-01PF-01

Fig. 6: Microscopic observations of GFP expression in A549 lung adenocarcinoma cells transfected with Viromer® ONE RED - Confocal microscopy

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

A549: transfection of plasmid DNA and mRNA

VR-01PF-01

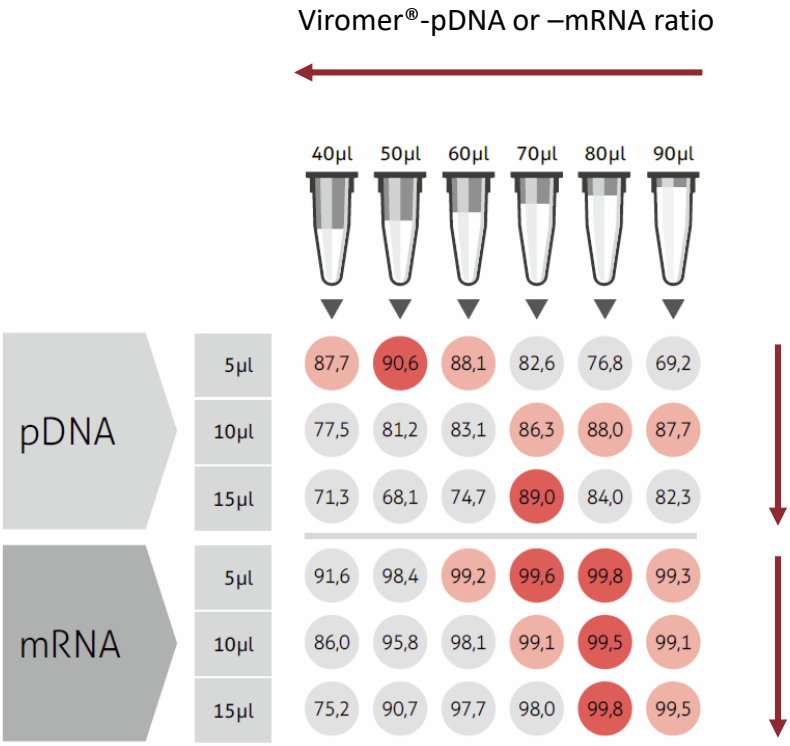


Fig. 7: Optimization of pDNA and mRNA transfections in A549 lung adenocarcinoma cells transfected with Viromer® ONE RED

- Numbers in circle corresponds to % of positive cells 24h post-transfection of a GFP plasmid (3.5kb) or a GFP encoding mRNA (996nt)
- Cells seeded 1 day before at $5x10^3$/96-well
- Test of 6 different rehydration volumes of the Viromer® vials (standard protocol: 80µl) to vary the Viromer-pDNA or –mRNA ratio,
- And 3 different transfer volumes (standard protocol: 10µl/96-well) to vary the amount of transfection complex arriving onto the cells

In-house data Lipocalyx GmbH