primary human monocyte-derived macrophages: transfection of siRNA

Fig. 1 and 2: Examples of western-blots showing high knockdown efficiency in human monocyte-derived macrophages transfected with Viromer® BLUE

- M2-phenotype macrophages diff. from PBMCs with M-CSF
- Transfection 6h post-differentiation
- Read-out after 48h.

Data from S. Medunjanin, University Hospital Magdeburg (Germany)

Data from A. Zhelankin, Bukrinsky’s lab, The George Washington University (USA)
Fig 3: Knock-down of cGAS in monocyte-derived dendritic cells (moDC) and macrophages (GM- and M-Mϕ) using Viromer® BLUE
Fig 7A from Paijo et al. PLoS Pathog. 2016: 12(4)

MATERIALS and METHODS

For transfection of cells with synthetic cGAMP (InvivoGen) or siRNA directed against cGAS (SMARTpool: siGENOME MB21D1, GE Healthcare), or control (siGENOME Non-Targeting siRNA Pool #2, GE Healthcare), the Viromer BLUE Kit (Lipocalyx) was used.

Final concentrations of 43 nM siRNA were packed in 22 μM Viromer following the manufacturer’s instructions and incubated with the cells for 72 h starting on day 2 after monocyte isolation and differentiation. 48 h post transfection and 1 h prior to stimulation differentiation medium was exchanged.

Transfection of 3 μg/ml synthetic cGAMP packed in 22 μM Viromer was performed similarly and cells were analyzed after 12 h by qPCR and supernatants were analyzed by ELISA after 24 h.


*cGAS Senses Human Cytomegalovirus and Induces Type I Interferon Responses in Human Monocyte-Derived Cells*

Institute for Experimental Infection Research, TWINCORE Hannover (Germany)
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Fig. 4: 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)

Data from M. Klöse, University Hospital Hamburg (Germany)
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Fig. 5: Knock-down of control and target genes in human monocyte-derived macrophages transfected with Viromer® BLUE or Viromer® GREEN

- monocyte-derived macrophages (differentiated from PBMCs by human serum)
- 6-well setup
- Donor 4 with Viromer® BLUE (50nM siRNA)
- Donor 3 with Viromer® GREEN (50nM siRNA)

Anonymous Data
primary human monocyte-derived macrophages: transfection of siRNA

Viromer® GREEN gave higher KDs than Viromer® BLUE
increasing efficiency from 12.5 to 50 nM siRNA
(25 nM corresponds to the standard protocol)

Data from A. Wortmann, Philipps-University Marburg (Germany)
primary human monocyte-derived macrophages: transfection of plasmid DNA

- Macrophage generation: Human PBMCs seeded on high-attachment cell culture dishes in RPMI 1640 medium with 2.5% human serum. Medium change every 2-3 days (do no re-seed floating cells). After 7 days, removal of all remaining non-attached cells.
- Before transfection, serum-starve macrophages for 24h
- Transfection protocol: standard conditions for 6-well (2 µg DNA/well). After complexation (15 min), adding of 9-fold amount of RPMI 1640 medium + 2.5% human serum to transfection mix, and transfer to wells.
- Incubation of transfection mix with cells overnight (~ 24 h)
- Perform individual experiments or harvest cells directly. Transfection stable for at least 72 h.

**Fig. 7:** Comparison of HA-tagged encoding protein expression in primary macrophages transfected with jetPEI® Macrophage and Viromer® RED

Data from A. Weigert, University Hospital – Institute of Biochemistry, Frankfurt am Main (Germany)
primary human monocyte-derived macrophages: transfection of mRNA

- transfection with pCMV-GFP plasmid and GFP-encoding mRNA (Viromer® RED Start Positive® Controls)
- FACS analysis
- Expression only observed with mRNA, no signal with plasmid DNA

Max. efficiency: 55% GFP+ cells with 750ng mRNA/24-well

Fig. 8: Comparison of GFP expression in primary macrophages transfected with plasmid or mRNA by using Viromer® RED

Anonymous Data
primary human monocyte-derived macrophages: transfection of mRNA

Fig. 9: Delivery of Cy3b-tagged (magenta) GFP-encoding mRNA into human monocyte-derived macrophages using Viromer® RED

“The ability of the particles to get into and transact macrophages in particular is impressive and appears markedly more efficient than Lipofectamine run in parallel”

Data from D. Russell, Cornell University (USA)
primary human monocyte-derived macrophages: transfection of mRNA

- 24-well plate format, standard protocol (500ng mRNA/well)
- highly efficient transfection of GFP-mRNA
- no signal with plasmid DNA (data not shown)

Fig. 10: Microscopic observations of GFP expression in human monocyte-derived macrophages transfected with Viromer® RED
GFP-mRNA Positive Control

Data from A. Wortmann, Philipps-University Marburg (Germany)