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Transfection Protocols ROTI®Fect plus – CL21

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Cells / Cell line: B16F10

A)

Cell density	24wells, 8x10^4 cells / ml, 0.5 ml medium, if grown w/o transfection: 70 % (90 %) confluency after 24 h (48 h)
Medium	RPMI + 10 % FBS + PenStrep
DNA / RNA	GFP expression plasmid pmaxFP-Green
Transfection complex	0.5 Plasmid-DNA + 0.5 / 1 / 2 / 4 µl ROTI®Fect plus in 50 µl PBS each for 20 mins.
Transfection	within 1 hour after seeding in 0.5 ml full medium
Incubation	in medium 24 h (37 °C, 5 % CO_2)
Detection	after 24 and 48 h by fluorescence microscopy
Result	24 h post transfection: Best transfection efficiency (18 %) with ratio 1:4 (0.5 g / 2 µl ROTI®Fect plus) 48 h post transfection: Best transfection efficiency (17 %) with ratio 1:8 (0.5 g / 4 µl ROTI®Fect plus) (rate with ratio 1:4 was lowered to 10 %) Virtually no cell toxicity (< 2 %)

Cells / Cell line: COLO205 (Human Colon Carcinoma)

A)

Cell density	12wells, 2x10^5 cells / ml, 24 h prior to transfection
Medium	x
DNA / RNA	Plasmid with GFP
Transfection complex	1,1 µg Plasmid-DNA + 50 µl serum free medium + 3 µl ROTI®Fect plus in serum free medium Formation of transfection complex for 20 mins. at ambient temp.
Transfection	in 1 ml medium + FCS over night
Incubation	in full medium (37 °C, 5 % CO_2) for 48 hours overall
Detection	FACS and fluorescence microscopy
Result	ROTI®Fect plus is well tolerated by the cell line with very low to no toxicity. (Fugene HD, used in parallel with the same parameters, did show significant toxicity.) Transfection rate was 2.6 %. Opinion is that this rate could be much higher if protocol would be optimised.

Cells / Cell line: COS7

A) Transfection of large population of cells in suspension

Seeding	Seeding of frozen COS7 cells in 75 cm^2 flasks, density $4x10^6$ cells per flask in 10 ml DMEM / 10 % FCS + antibiotics (geneticin). Incubate for 2-3 days. They trypsinate the cells (Hanks' BSS + EDTA + trypsine) 10 m in. RT, Pellet by centrifugation at 300 g for 10 mins at RT. Resuspend pellet in DMEM/10% FCS, count and adjust cells to 2-2.5 x 10^6 cells / ml.
Medium	DMEM incl. 10 % FBS + antibiotics / OPIMEM
DNA / RNA	pEGFP
Transfection complex	ROTI®Fect plus : DNA in a ratio of 2:1, preincubated for 20 mins in OPTIMEM
Transfection	Combine 2 ml cell suspension with 100 µl transfection mix
Incubation	Incubate in vials under shaking (400 rpm) for 6-8 hours under CO_2 atmosphere. Then plate dilute with DMEM and plate for analysis/ assays.
Detection	Fluorescence microscopy
Result	Typical transfection efficiency: 60 %. Well reproducible.

B)

Seeding	6wells, $2.5x10^5$ cells / well, 24 hrs prior to transfection
Medium	DMEM incl. FBS + antibiotics / OPTIMEM
DNA / RNA	pCS2-GFP construct
Transfection complex	dilute ROTI®Fect plus 12 µl in 250 µl serum-/antiobiotics-free OPTIMEM dilute DNA 4 µg in 250 µl serum-/antiobiotics-free OPTIMEM --> incubate separately for 5 mins at RT combine and incubate at RT for 20-30 mins.
Transfection	Wash cells 2x with OPTIMEM, overlay with 1.5 ml pure OPTIMEM. Add transfection complex, rock gently for even distribution. Incubate for 4-6 hrs.
Incubation	Replace transfection medium by 2 ml full DMEM and incubate for 24 hours.
Detection	Assay by either flow cytometry or confocal microscopy.
Result	Comparison with lipofectamine 2000: ROTI®Fect plus was at least as efficient as lipofectamine. For downstream experiments, solely ROTI®Fect plus was used.

C)

Seeding	12wells, $2x10^5$ cells / well, prior to transfection, incubate overnight -> 80-90 % confluency.
Medium	RPMI-1640 incl. 10 % FBS + antibiotics (amphot., pen. strept.) / OPTIMEM
DNA / RNA	pEGFP construct
Transfection complex	Dilute ROTI®Fect plus with 100 µl OPTIMEM, incubate for 15 mins at RT. Then add DNA as follows: ROTI®Fect plus : DNA in ratios of (µl : µg) 1:0.5, 2:0.5, 4:0.5, 6:0.5, 2:1, 4:1, 8:1, 12:1, 4:1.5, 8:1.5, 12:1.5 or 16:1.5 incubate mix at RT for 15 mins.
Transfection	Wash cells 2x with OPTIMEM, overlay with 1 ml pure OPTIMEM. Add transfection complex and mix gently.
Incubation	Incubate for 24 hours.
Detection	Wash cells with PBS and assay by confocal fluorescence microscopy.
Result	All ratios showed transfection. Best results (80-95 % transfection efficiency) were obtained with ratios of: 1:0.5, 2:0.5, 4:0.5, 6:0.5, 8:1, 12:1 and 12:1.5

Cells / Cell line: HCT116 (Human colon carcinoma)

A)

Cell density	12wells, 2x10^5 cells / well, 24 hours prior to transfection
Medium	unknown
DNA / RNA	Plasmid with GFP
Transfection complex	1,1 µl (1 µg DNA/µl) Plasmid-DNA in 50 µl serum free medium + 3,0 µl ROTI®Fect plus in 50 µl serum-free medium Mix and incubate for formation of transfection complex for 20 mins.
Transfection	add 1 ml fresh medium (incl. 10 % FCS) to the cells, add transfection complex. Incubate over night
Incubation	Change medium after approx. 12-14 hours, incubate for two days overall in full medium (37 °C, 5 % CO_2)
Detection	Fluorescence microscopy, FACS analysis
Result	Very good transfection rate of nearly 40 %, much higher than the rate achieved with Fugene HD (13 %). No or only low toxicity.

B)

Cell density	6wells, 1,5x10^5 cells / well, 48 hours prior to transfection
Medium	DMEM, 10 % FCS, Pen/Strep, HEPES
DNA / RNA	Plasmid with GFP
Transfection complex	1 µg DNA Plasmid-DNA in 50 µl serum/antib. free medium + 2, 4, 6 µl ROTI®Fect plus in 50 µl serum/antib. free medium Mix and incubate for formation of transfection complex for 15 mins.
Transfection	add transfection complex to the cells (with / w/o medium?). Incubate for 5 h.
Incubation	Change medium after 5 hours, incubate 24 h in full medium (37 °C, 5 % CO_2)
Detection	Fluorescence microscopy
Result	Good transfection rate with 4 µl ROTI®Fect plus. Low toxicity. However, for other cell lines 2 or 6 µl ROTI®Fect plus gave better results. Amounts must be optimised for each cell line.

Cells / Cell line: HEK293

A) siRNA

Seeding	96wells, 1.4x10^4 cells / well, 24 hrs prior to transfection
Medium	DMEM incl. 10 % FBS
DNA / RNA	siRNA labelled with AlexaFluor647
Transfection complex	ROTI®Fect plus 1 7 2 / 3 / 4 µl : siRNA 2 / 5 / 8 nM in 20 µl serum-/antiobiotics-free medium for 15 mins.
Transfection	24 h in 120 µl medium
Incubation	-
Detection	Fluorescence plate reader
Result	best results with 2, 3, and 4 µl ROTI®Fect plus: 8 nM siRNA. Cell viability best for 1-2 µl ROTI®Fect plus. ➔ Best results with 2 µl ROTI®Fect plus / 8 nM siRNA

B)

Seeding	6wells, 3.5x10^5 cells / well, 24 hrs prior to transfection, confluency at transfection 80-90 %
Medium	DMEM incl. 10 % FBS + antibiotics
DNA / RNA	pGFP
Transfection complex	ROTI®Fect plus 2 / 4 / 8 µl : DNA 1 / 2 µg in 500 µl serum-/antiobiotics-free OPTI-MEM for 20 mins.
Transfection	Plus cells -> 5 h
Incubation	in DMEM incl. 10 % FBS, time?
Detection	Microscope: Fluorescence and cell viability
Result	Best result (100 % transfection) with ROTI®Fect plus : DNA 8 µl : 2 µg, but also high cell death rate. Very good result (70-80 % tr.) with ROTI®Fect plus : DNA 4 µl : 1 or 2 µg. Only few cells dead.

C)

Seeding	6wells, ? cells / well, ? hrs prior to transfection, confluency at transfection 80 %
Medium	?
DNA / RNA	pGFP
Transfection complex	ROTI®Fect plus 4, 6, 8, 12, 16 µl : DNA 1, 2, 4, 6 µg in 200 µl serum-/antiobiotics-free OPTI-MEM for 20 mins.
Transfection	Plus cells -> 4 h
Incubation	In medium for 24 or 48 h
Detection	Microscope
Result	best result (90 % transfection) with ROTI®Fect plus : DNA 24 µl : 4 µg. Also good results (60-70 %) with other constructs at the same ratio.

D)

Seeding	10 cm petri dishes, ? cells / dish, 24-48 hrs prior to transfection, confluency at transfection 60-80 %
Medium	DMEM incl. 10 % FBS + antibiotics
DNA / RNA	pGABA receptor
Transfection complex	ROTI®Fect plus 28, 42, 56, 112, 168 µl : DNA 14 µg in 250 µl serum-/antiobiotics-free OPTI-MEM for 15 mins.
Transfection	Plus cells -> ? h
Incubation	in DMEM incl. 10 % FBS, 48 h?
Detection	Ligand binding assay
Result	best result (100 % transfection) with 3 and 4 µl ROTI®Fect plus per µg DNA

E) Reverse Transfection Cell Microarray (RTCM) on glass slides

Seeding	384well plates, 1x10^5 cells / cm^2, seeded after application of the transfection complex, confluency after 48 h
Medium	DMEM incl. 10 % FBS + antibiotics
DNA / RNA	pGFP
Transfection complex	ROTI®Fect plus 2, 3, 4 µl DNA 1, 2, 3, 4 µg mix DNA and ROTI®Fect plus add 3 µl serum-/antibiotics-free OPTI-MEM incl. 0.4 M sucrose add water to final vol. of 11.5 µl incubate for 20 mins.
Transfection	Mix transfection complex sol. with 7.25 µl 0.2 % gelatine and transfer to 384well plate with cells. Incubation time unknown.
Incubation	For 48 h in medium
Detection	Laser scan
Result	Very good result of 80 % transfection efficiency with ROTI®Fect plus : DNA of 3:1, 4:1 or 4:2. Expression rate was higher after transfection with ROTI®Fect plus compared to transfections with Lipofectamine 2000, but more dependent on the ROTI®Fect plus / DNA ratio used.

F) Transfection of large population of cells in suspension

Seeding	Seeding of frozen HEK293 cells in 75 cm^2 flasks, density 4x10^6 cells per flask in 10 ml DMEM / 10 % FCS + antibiotics (geneticin). Incubate for 2-3 days. They trypsinate the cells (Hanks' BSS + EDTA + trypsine) 10 m in. RT, Pellet by centrifugation at 300 g for 10 mins at RT. Resuspend pellet in DMEM/10% FCS, count and adjust cells to 2-2.5 x 10^6 cells / ml.
Medium	DMEM incl. 10 % FBS + antibiotics / OPIMEM
DNA / RNA	pEGFP
Transfection complex	ROTI®Fect plus : DNA in a ratio of 2:1, preincubated for 20 mins in OPTIMEM
Transfection	Combine 2 ml cell suspension with 100 µl transfection mix
Incubation	Incubate in vials under shaking (400 rpm) for 6-8 hours under CO_2 atmosphere. Then plate dilute with DMEM and plate for analysis/ assays.
Detection	Fluorescence microscopy
Result	Typical transfection efficiency: 60 %. Well reproducible.

Cells / Cell line: HeLa

A)

Seeding	12wells, 2x10^5 cells / well, 24 hrs prior to transfection (50 % confluency)
Medium	DMEM incl. 10 % FCS
DNA / RNA	GFP-tagged gap junction protein
Transfection complex	ROTI®Fect plus: DNA 1:1; 2:1; 4:1; 6:1 in ?? µl serum-/antiobiotics-free DMEM medium for ?? mins.
Transfection	?
Incubation	?
Detection	?
Result	best results with 2 µl ROTI®Fect plus: 1 µg DNA

B)

Seeding	6wells, 4x10^5 cells / well, 4-6 hrs prior to transfection
Medium	?
DNA / RNA	snRNA
Transfection complex	ROTI®Fect plus: RNA 2:1; 3:1; 4:2; 6:2 in ?? µl serum-/antiobiotics-free DMEM medium for ?? mins.
Transfection	?
Incubation	48 hrs
Detection	FISH
Result	best results with 6 µl ROTI®Fect plus: 2 µg RNA

C)

Seeding	48wells, 1.5x10^5 cells / well, 24 hrs prior to transfection (60 % confluency)
Medium	DMEM incl. 10 % FBS
DNA / RNA	CMV Promotor regulated Luciferase
Transfection complex	ROTI®Fect plus: DNA 1:0.5; 2:0.5; 2:1; 4:1 in 100 µl serum-/antiobiotics-free DMEM medium for 15 mins without and then 15 mins. with DNA.
Transfection	4 hrs.
Incubation	in DMEM incl. 10 % FBS, 48 hrs
Detection	Luciferase assay
Result	best results with 2 µl ROTI®Fect plus: 1 µg DNA

D)

Seeding	24wells, 10^5 cells / well, 24 hrs prior to transfection (60 % confluency)
Medium	DMEM incl. 10 % FCS
DNA / RNA	Flag-tagged version of the Salmonella effector in plasmid
Transfection complex	ROTI®Fect plus: DNA 0.5:0.25; 1:0.25; 2:0.25; 3:0.25; 1:0.5; 2:0.5; 4:0.5; 6:0.5; 2.1; 4:1; 6:1; 8:1; in 30 µl serum-/antiobiotics-free DMEM medium for 17 mins.
Transfection	4 hrs.
Incubation	in DMEM incl. 10 % FBS, 24 hrs
Detection	Western-Blot
Result	best results with 6 µl ROTI®Fect plus: 0.5 µg DNA or 8µl : 1 µg DNA

E) Reverse Transfection Cell Microarray (RTCM) on glass slides

Seeding	384well plates, 2,5x10^4 cells / cm^2, seeded after application of the transfection complex, confluency after 48 h
Medium	DMEM incl. 10 % FCS + PenStrep
DNA / RNA	pGFP
Transfection complex	ROTI®Fect plus 2, 3, 4 µl DNA 1, 2, 3, 4 µg mix DNA and ROTI®Fect plus add 3 µl serum-/antibiotics-free OPTI-MEM incl. 0.4 M sucrose add water to final vol. of 11.5 µl incubate for 20 mins.
Transfection	Mix transfection complex sol. with 7.25 µl 0.2 % gelatine and transfer to 384well plate with cells. Incubation time unknown.
Incubation	For 48 h in medium
Detection	Laser scan
Result	Best results with 3 or 4 µl ROTI®Fect plus: 1 µg DNA. Higher efficiency than Lipofectamine 2000.

F)

Seeding	6wells, ? cells / well, 24 hrs prior to transfection (70 % confluency)
Medium	DMEM incl. 10 % FCS
DNA / RNA	mitochondria targeted probe, GFP
Transfection complex	ROTI®Fect plus: DNA 3:1 or 3:1.5 in ?? µl serum-/antiobiotics-free DMEM medium for ?? mins.
Transfection	?
Incubation	in DMEM incl. 10 % FBS, ?
Detection	Fluorescence microscopy
Result	approx. 70 % transfection efficiency with both approaches. Mitochondria were suffering, however.

G)

Seeding	6wells, ? cells / well
Medium	DMEM incl. 10 % FCS
DNA / RNA	GFP-Tagged protein, plasmid
Transfection complex	ROTI®Fect plus: DNA 3:1 or 4:1 in ?? µl serum-/antiobiotics-free DMEM medium for ?? mins.
Transfection	?
Incubation	in DMEM incl. 10 % FBS, 24 hrs
Detection	Microscopy and FACS
Result	best results with 3 µl ROTI®Fect plus: 1 µg DNA

H)

Seeding	6wells, ? cells / well, 24 hrs prior to transfection (50 % confluency)
Medium	DMEM incl. 10 % FBS
DNA / RNA	siRNA against ubiquitin ligase
Transfection complex	ROTI®Fect plus: RNA 6:5; 12:5; 18:5 in 200 µl serum-/antiobiotics-free Opti-mem medium for 25 mins.
Transfection	4 hrs.
Incubation	in DMEM incl. 10 % FBS, 48 hrs
Detection	Western-Blotting
Result	best results with 18 µl ROTI®Fect plus: 5 µg RNA, Very efficient in stabilizing the siRNA targets.

I)

Seeding	24wells, 2x10^5 cells / well, 24 hrs prior to transfection (50 % confluency)
Medium	Optimem incl. 10 % FCS
DNA / RNA	GFP-tagged protein, plasmid
Transfection complex	ROTI®Fect plus: DNA 1:1 in 100 µl serum-/antiobiotics-free Optimem medium for 15 mins.
Transfection	4-6 hrs in 200 µl medium in total
Incubation	in Optimem incl. 10 % FBS, 48 hrs
Detection	Fluorescence microscopy
Result	Very good results. Same efficiency as Lipofectamine 2000. Less cytotoxicity then ROTI®-Fect.

Cells / Cell line: HepG2

A)

Seeding	6wells, 2x10^5 cells / well, 24 hrs prior to transfection, confluency 60-80 %
Medium	RPMI incl. 10 % FBS + antibiotics
DNA / RNA	Plasmid
Transfection complex	ROTI®Fect 1.5 - 9 µl : DNA 2µg in 200 µl serum-/antiobiotics-free medium for 20 mins.
Transfection	5 hrs. in 1 ml RPMI serum-free
Incubation	in RPMI incl. 20 % FBS, 48 hrs
Detection	Western-Blotting
Result	Best results with 3 µl ROTI®Fect plus : 2 µg DNA

B)

Seeding	24wells, ? cells / well, 24 hrs prior to transfection, confluency 50 %
Medium	DMEM incl. 10 % FBS + antibiotics
DNA / RNA	GFP-plasmid
Transfection complex	ROTI®Fect 0.25 - 2 µl : DNA 2µg in 50 µl serum-/antiobiotics-free medium for 20mins. and: ROTI®Fect 0.5 µl : DNA 0.5 – 6 µg in 50 µl serum-/antiobiotics-free medium for 20mins.
Transfection	? hrs.
Incubation	in DMEM incl. 10 % FBS, 24 hrs
Detection	Fluorescence microscope
Result	Best results with 0.5 µl ROTI®Fect plus : 2 µg DNA

Cells / Cell line: HUVEC (Human umbellical vein endothelial cells)

A)

Cell density	gelatine-coated 48wells, 5x10^4 cells / well, >50 % true confluency (24 h)
Medium	RPMI-1640 plus 10 % human serum, antibiotics, glutamine
DNA / RNA	GFP-plasmid
Transfection complex	0.1 µg DNA in 15 µl RPMI (ser- and antibiot.-free) 0.5 µl ROTI®Fect plus in 10 µl RPMI (ser- and antibiot.-free) Mix and incubate 20 mins. at RT
Transfection	in 250 µl RPMI (w/o serum / antibiotics?) 12-16 hrs
Incubation	replace by fresh RPMI (full) and incubate 24 and 48 h (37 °C, 5 % CO_2)
Detection	Fluorescence microscopy and flowcytometry
Result	Only a small percentage of cells (approx. 4 %) expresses high amounts of GFP, but a considerable number (n.d.) expresses GFP on a low level. Transfection was successful, but may be optimised.

Cells / Cell line: Jurkat (T-lymphoblastic leukaemia cell line)

A)

Cell density	12well culture plate, 10^5 cells / well (24 h)
Medium	RPMI-1640 plus 10 % human serum, antibiotics, glutamine, HEPES cells were washed before transfection and seeded in 0.9 ml OptiMEM
DNA / RNA	GFP-plasmid, CMV-promoter
Transfection complex	1.5 µg DNA in 50 µl OptiMEM (ser- and antibiot.-free) 3 µl ROTI®Fect plus in 50 µl OptiMEM (ser- and antibiot.-free) Mix and incubate 20 mins. at RT
Transfection	in 900 µl OptiMEM (w/o serum / antibiotics?) for 5 hrs.
Incubation	replace by fresh RPMI (full) and incubate 48 h (37 °C, 5 % CO_2)
Detection	flowcytometry
Result	Assays with ROTI®Fect plus resulted in higher transfection rates (33 %), compared to transfections with Dharmafect and Lipofectamin 2000. No toxic effects whatsoever.

B)

Cell density	12well culture plates, 4x10^5 cells / well (24 h), 1 ml medium
Medium	RPMI-1640 plus 10 % FCS, antibiotics
DNA / RNA	Several plasmids, incl. β-galactosidase gene and luciferase as positive controls.
Transfection complex	2 / 3 / 4 / 8 / 12 / 16 µl ROTI®Fect plus in 100 µl sterile PBS then add 0.5 / 1 / 1.5 µg DNA incubate 15 mins. at RT
Transfection	in 1 ml RPMI (w/o serum / antibiotics?) for 24 hrs.
Incubation	24 hrs.
Detection	LucLite plus or β-galactosidase assay
Result	Highest expression of luciferase was obtained with 16 µl ROTI®Fect plus : 1.5 µg DNA. Transfection with β-galactosidase also was very successful with this ratio. Cell viability was over 98 %.

Cells / Cell line: KELLY (human neuroblastoma)

A)

Cell density	48wells, 10^5 cells / 1 ml, seeded 1 day prior to transfection, used at 70-80 % confluency
Medium	RPMI 1640, 10% FCS, L-Glu, Pen/Strep
DNA / RNA	Plasmid, red fluorescence
Transfection complex	Plasmid-DNA : ROTI®Fect plus as 1 µl : 0.5 µg (2.1) / 2 µl : 0.5 µg (4:1) / 2 µl : 1 µg (2:1) in 100 µl medium for 15min
Transfection	in 0.9 ml RPMI (-> final 1 ml)
Incubation	in medium 72 h (37 °C, 5 % CO_2)
Detection	FACS, measurement of cell viability
Result	Highest percentage of transfected cells (18 %) was obtained using 2 µl ROTI®Fect plus : 1 µg DNA. However, percentage of viable cells was lowest, approx. 30 %. Ratio of 4 :1 (2 µl : 0.5 µg DNA) was a bit less efficient in transfection rates (12 %), but also much less toxic (approx. 60 % viable cells). Overall, ratio of 4:1 (2 µl ROTI®Fect plus : 0.5 µg DNA gave best results.

Cells / Cell line: LS174T (Human Colon Carcinoma)

A)

Cell density	12wells, 2x10^5 cells / ml, 24 h prior to transfection
Medium	x
DNA / RNA	Plasmid with GFP
Transfection complex	1,1 µg Plasmid-DNA + 50 µl serum free medium + 3 µl ROTI®Fect plus in serum free medium Formation of transfection complex for 20 mins. at ambient temp.
Transfection	in 1 ml medium + FCS over night
Incubation	in full medium (37 °C, 5 % CO_2) for 48 hours overall
Detection	FACS and fluorescence microscopy
Result	ROTI®Fect plus is well tolerated by the cell line with very low to no toxicity. (Fugene HD, used in parallel with the same parameters, did show significant toxicity.) Transfection rate was 28.2 %. (Fugene HD: 19.3 %)

Cells / Cell line: MCF-7 (Michigan Cancer Foundation – 7, Human Breast Carcinoma)

A)

Cell density	12wells, 2x10^5 cells / ml, 24 h prior to transfection
Medium	x
DNA / RNA	Plasmid with GFP
Transfection complex	1,1 µg Plasmid-DNA + 50 µl serum free medium + 3 µl ROTI®Fect plus in serum free medium Formation of transfection complex for 20 mins. at ambient temp.
Transfection	in 1 ml medium + FCS over night
Incubation	in full medium (37 °C, 5 % CO_2) for 48 hours overall
Detection	FACS and fluorescence microscopy
Result	ROTI®Fect plus is well tolerated by the cell line with very low to no toxicity. (Fugene HD, used in parallel with the same parameters, did show significant toxicity.) Transfection rate was 1.4 %. Opinion is that this rate could be much higher if protocol would be optimised.

Cells / Cell line: MEF (Murine embryonic fibroblasts)

A)

Cells	1x10^5 cells / ml
Medium	OPTI-MEM®
DNA / RNA	plasmid construct or siRNA
Transfection complex	1 µg DNA + 5 µl ROTI®Fect plus in 50 µl medium
Transfection	with 2 ml cells for 24 or 48 hrs.
Detection	Immunostaining
Result	Good results with both, plasmid and siRNA. Also good in simultaneous knock-down transfection.

B)

Cells	0.67x10^5 cells / 12well for 5 hrs.
Medium	OPTI-MEM®
DNA / RNA	his-flag tagged expression vector
Transfection complex	1.5 µg DNA + 2.5 µl ROTI®Fect plus in 100 µl medium or PBS
Transfection	20 hrs. in full medium
Detection	Western-Blot
Result	Higher expression level with using ROTI®Fect plus than with Lipofectamine 2000 or Fugene HD.

C)

Seeding	to 60 % confluency in 12 wells 2 hrs prior to transfection
Medium	DMEM incl. 10 % FBS
DNA / RNA	GFP-plasmid construct
Transfection complex	1 and 1.5 µg DNA + 1 - 12 µl ROTI®Fect plus in 100 µl serum-free medium
Transfection	in 1 ml medium incl. 5 % FBS, 6 hrs.
Incubation	full medium
Detection	cell viability, fluorescence microscopy
Result	best results with 1 µg DNA and 4 or 10.5 µl ROTI®Fect plus or with 1.5 µg DNA and 10.5 µl ROTI®Fect plus. However, toxic effects were higher when using 10.5 µl ROTI®Fect plus. Best use 1µg DNa / 1 µl ROTI®Fect plus

Cells / Cell line: mouse neuroblastoma cells

A) Neuro 2A, mouse neuroblastoma

Seeding	24wells with cover slips, 8.0 x 10^4, 8.0 x 10^5 cells / well, 1 day hrs. prior to transfection
Medium	DMEM incl. 10 % FBS + antibiotics
DNA / RNA	Plasmid, recombinant, GFP
Transfection complex	DNA : ROTI®Fect plus 0.5 : 2.0 and 1.0 : 4.0 µg:µl in serum-/antibiotics-free medium
Transfection	20 hrs.
Incubation	x
Detection	microscopy of fixated cells
Result	Results were compared between transfection with ROTI®Fect plus, Lipofectamine 2000, DOTAP and FuGENE 6. Best results with 8.0 x 10^4 cells. Only low transfection rates with Lipofectamine, FuGene 6 and DOTAP (ca. 5 %), very good transfection rate with ROTI®Fect plus (ca. 60 %). Best DNA : ROTI®Fect ratio (µg/µl) 0.5:2

B) N18TG2, mouse neuroblastoma

Cell density	24wells + cover slip, 8 x 10^4 cells / well, incubation ca. 1 day. Replace medium for 2 hrs, then transfect.
Medium	DMEM + FCS (10 %) + Antibiotics
DNA / RNA	Plasmid, recombinant, GFP
Transfection complex	ROTI®Fect plus: 1 µl / 1.5 µl / 2 µl in 30 µl PBS DNA: 0.5 µg in 30 µl PBS mix by pipetting incubate for 20 mins at RT
Transfection	add to cells plus medium
Incubation	12 or 36 hrs
Detection	Microscopy
Result	Considering both (efficiency and cytotoxicity), optimal DNA / ROTI®Fect plus ratio is 0.5 µg : 2 µl for 36 hrs (over 50 %). Low cell toxicity.

C) 2/4, mouse neuroblastoma

Cell density	24wells + cover slip, 8 x 10^4 cells / well, incubation ca. 1 day. Replace medium for 2 hrs, then transfect.
Medium	DMEM + HEPES + sodium bicarbonate + FCS (10 %) + Antibiotics
DNA / RNA	Plasmid, recombinant, GFP
Transfection complex	ROTI®Fect plus: 1 µl / 1.5 µl / 2 µl in 30 µl PBS DNA: 0.5 µg in 30 µl PBS mix by pipetting incubate for 20 mins at RT
Transfection	add to cells plus medium
Incubation	12 or 36 hrs
Detection	Microscopy
Result	Considering both (efficiency and cytotoxicity), optimal DNA / ROTI®Fect plus ratio is 0.5 µg : 1.5 µl for 36 hrs (nearly 30 %). Very low cell toxicity.

Cells / Cell line: NIH3T3 (Murine ebryonic fibroblast)

A)

Seeding	6wells
Medium	DMEM incl. 10 % FCS
DNA / RNA	plasmid construct
Transfection complex	1 µg DNA + 3 or 4 µl ROTI®Fect plus in DMEM incl. 10 % FCS
Transfection	?
Incubation	in DMEM incl. 10 % FCS, 24 hrs
Detection	FACS
Result	Very good results with both DNA amounts. ROTI®Fect plus had best ratio transfection efficiency / toxicity (test of 4 reagents overall) Comparison with lipofectamine 2000: Lipofectamine had higher transfection efficiency, but also higher toxicity.

B)

Seeding	48wells, 1.5x10^5 cells / well, 24 hrs prior to transfection
Medium	DMEM incl. 10 % FBS
DNA / RNA	Luc-plasmid construct under CMV promotor
Transfection complex	ROTI®Fect plus: DNA 2:1 or 4:1 in 100 µl serum-/antiobiotics-free medium for 15 mins. without and 15 mins. with DNA.
Transfection	4 hrs.
Incubation	in DMEM incl. 10 % FBS, 48 hrs
Detection	Luciferase assay system (RLU) and cell viability
Result	best results with 2 µl ROTI®Fect plus: 1 µg DNA. Use no less than 2 µl ROTI®Fect plus.

C)

Seeding	8wells, 3x10^5 cells / well, 16 hrs prior to transfection
Medium	DMEM incl. FCS
DNA / RNA	plasmid construct
Transfection complex	0.14 µg DNA + 1.4 µl ROTI®Fect plus in 27 µl serum-/antiobiotics-free medium
Transfection	in 300 µl full medium
Incubation	in DMEM incl. FCS, 48 hrs
Detection	Immunofluorescence
Result	good results with ROTI®Fect (efficiency > 5 %) Comparison with lipofectamine 2000: Transfection efficiency was 5times higher with ROTI®Fect plus than with Lipofectamine (approx. 0.1 % efficiency)

D)

Seeding	6wells, 5x10^5 cells / well, 24 hrs prior to transfection
Medium	DMEM incl. 10 % FCS
DNA / RNA	Luc-plasmid construct under CMV promotor
Transfection complex	3 µl ROTI®Fect plus + 1 µg DNA in 200 µl PBS
Transfection	6 hrs. in 2 ml medium incl. FCS (without antibiotics)
Incubation	in DMEM incl. 10 % FCS, 48 hrs
Detection	Luciferase assay system (RLU)
Result	Transfection efficiency approx. 20 %, over 15 x higher than with Transfast reagent.

Cells / Cell line: Panc-1 (Human epithelioid carcinoma cell line)

A)

Cell density	12wells, 2x10^5 cells / ml, 24 h prior to transfection
Medium	x
DNA / RNA	Plasmid with GFP
Transfection complex	1,1 µg Plasmid-DNA + 50 µl serum free medium + 3 µl ROTI®Fect plus in serum free medium Formation of transfection complex for 20 mins. at ambient temp.
Transfection	in 1 ml medium + FCS over night
Incubation	in full medium (37 °C, 5 % CO_2) for 48 hours overall
Detection	FACS and fluorescence microscopy
Result	ROTI®Fect plus is well tolerated by the cell line with very low to no toxicity. (Fugene HD, used in parallel with the same parameters, did show significant toxicity.) Transfection rate was 11.5 %. (Fugene HD 2 %)

Cells / Cell line: PC12 (rat adrenal medulla, neuroendocrine)

A)

Cell density	4wells + cover slip, 1-2 x 10^6 cells / cover slip, incubation ca. 1 day, 90-100 % optical confluency
Medium	Opti-MEM DMEM
DNA / RNA	Plasmids, recombinant pCR-CMV and pCDNA3 with FLAG and GFP-Tags
Transfection complex	0,5-1,5 µg DNA in 50 µl medium w/o serum/antibiot. 1.0 – 6.0 µl ROTI®Fect plus in 50 µl medium w/o serum/antibiot. mix by pipetting incubate for 15-20 mins.
Transfection	add to cells and incubate for 6 hrs.
Incubation	add 260 µl DMEM + serum incubate in medium overnight (37 °C, 5 % CO_2) change medium to pure DMEM with serum
Detection	Microscopy
Result	Transfection as good as with Lipofectamine and Fugene 6. No significant cytotoxicity. Also big vector / insert constructs (up to 19 kb) may be transfected efficiently.

B)

Seeding	24wells with cover slips, 1.0 x 10^4, 7.0 x 10^4, 2.0 x 10^5 cells / well, 1 day hrs. prior to transfection
Medium	DMEM incl. 10 % FBS + antibiotics
DNA / RNA	Plasmid recombinant, GFP
Transfection complex	DNA 0.8 and 1.0 µg ROTI®Fect plus 2.0 and 4.0 µl in serum-/antibiotics-free medium
Transfection	20 hrs.
Incubation	x
Detection	microscopy of fixated cells
Result	Results were compared between transfection with ROTI®Fect plus, Lipofectamine 2000 and FuGENE 6. Best results with 2.0 x 10^5 cells and DNA : ROTI®Fect plus ratio (µg/µl) of 1:4 (ca. 30 % of cells, ROTI®Fect plus resulted in stronger fluorescence -> higher copy number).

Cells: primary glioma cells

A) Human glioma cells

Cell density	48wells, 2x10^4 cells / well, 60 % confluency
Medium	DMEM + 10 % FCS + streptomycin.
DNA / RNA	pCMV-EGFP plasmid
Transfection complex	- 0.35 µg Plasmid-DNA in 20 µl medium (w/o serum and str.) - add 0.4 / 1 / 2 / 5 / 7 / 10 µl ROTI®Fect plus - incubate for 20 mins at RT. Then add to cells.
Transfection	4 hrs in 250 µl DMEM + FCS, then wash in full medium.
Incubation	in 0.5 m l full medium for 48 h (37 °C, 5 % CO_2)
Detection	FACS and inverted phase contrast microscopy (morphology)
Result	Best results with 1 and 2 µl ROTI®Fect plus (more or less identical results) Only very little cell cytotoxicity when using 7 µl ROTI®Fect plus or less. Virtually no cytotoxicity in siRNA assays.

Cells: primary hepatocytes

A)

Cell density	6wells, 4x10^5 cells / well (2 ml), 90 % confluency
Medium	DMEM or Opti-MEM + 10 % FBS. Both tested, both ok.
DNA / RNA	GFP-plasmid
Transfection complex	- 1, 2, 3, 4, or 5 µg Plasmid-DNA in 100 µl medium - x µl ROTI®Fect plus in 100 µl medium, so that the DNA (µg) : ROTI®Fect plus ratio is 1:0.5 / 1:1 / 1:2 / 1.4 / 1.8 / 1:16 - combine, incubate for 20 mins. Then add to cells.
Transfection	3 and 16 hrs
Incubation	in full medium for 24, 48 or 72 h (37 °C, 5 % CO_2)
Detection	Fluorescence illuminator
Result	Best results with ratio DNA:ROTI®Fect plus 1:4, best results with 2 µg Plasmid (hence 2 µg plasmid : 8 µl ROTI®Fect plus) Much less cytotoxic than Lipofectine 2000 after incubation for 48 or 72 hours. Cytotoxicity negligible. Transfection efficiency more than 90 %.

B) silencing assay

Cell density	6wells, 4x10^5 cells / well (2 ml), 90 % confluency
Medium	DMEM + 10 % FBS
DNA / RNA	miRNA or siRNA
Transfection complex	- 0.5, 1, or 2.5 µg miRNA in 100 µl medium - 1.5, 3, or 7.5 µl ROTI®Fect plus in 100 µl medium - combine, incubate for 20 mins. Then add to cells.
Transfection	? hrs
Incubation	in full medium for 24, 48 or 72 h (37 °C, 5 % CO_2)
Detection	Western-Blot
Result	Good silencing efficiency, negligible cytotoxicity.

Cells / Cell line: RAW264.7 (Murine macrophage cell line)

A) For transient transfection

Cell density	24wells, 1.2x10^5 cells / well, 70 % confluency
Medium	DMEM/F12 full medium
DNA / RNA	GFP plasmid
Transfection complex	4 µg Plasmid-DNA in 30 µl DMEM, 5 mins. at RT 4 / 6 / 8 µl ROTI®Fect plus in 30 µl medium, 5 mins. at RT Combine → then incubate 20 mins. at RT
Transfection	4 hrs
Incubation	in 1 ml fresh medium (DMEM/F12) 36 h (37 °C, 5 % CO_2)
Detection	Fluorescence microscopy
Result	Very good transfection result with ratio 4:6. Ratio 4:4 was less efficient and 4:8 became a bit toxic.

B) For stable transfection

Seeding	10 cm Petri dishes, 3.5x10^6 cells / dish, 24 hrs prior to transfection
Medium	DMEM/F12 full medium
DNA / RNA	cDNA made from a LUC construct (plasmid)
Transfection complex	ROTI®Fect plus 40 µl in 700 µl medium (ser./ant. free), 5 mins. at RT DNA 24µg in 700 µl medium (ser./ant. free), 5 mins. at RT Combine → then incubate 20 mins. at RT
Transfection	4 hrs.
Incubation	in full DMEM/F12 with 0.8 mg/ml G418
Detection	microscope
Result	Efficient transfection and stable clones. Good mRNA transcription. As efficient as transfection with lipofectamine 2000.

C) Co-transfection

Cell density	12wells, 0.5x10^6 cells / ml, 1 ml / well, cells were counted prior to seeding. Near 100 % viability.
Medium	DMEM full medium
DNA / RNA	LUC promoter-plasmid and construct with regulating gene (plasmid)
Transfection complex	1.5 µg LUC plasmid-DNA + 1 µg DNA of regulator construct in 100 µl medium (ser/ant free) 5 µl ROTI®Fect plus in 100 µl medium (ser/ant free) Combine → then incubate 20 mins. at RT
Transfection	4 hrs, 60 µl mix / well in 0.5 m l DMEM (ser/ant free)
Incubation	add 0.5 ml DMEM (full incl. 20 % FCS), 24 h (37 °C, 5 % CO_2)
Detection	Luminometer
Result	Cells only slightly stressed, very strong transactivation

Cells / Cell line: S91 (Murine melanoma cell line)

A)

Cell density	24wells, 8x10^4 cells / ml, 0.5 ml medium, if grown w/o transfection: 70 % (90 %) confluency after 24 h (48 h)
Medium	RPMI + 10 % FBS + PenStrep
DNA / RNA	GFP expression plasmid pmaxFP-Green
Transfection complex	0.5 Plasmid-DNA + 0.5 / 1 / 2 / 4 µl ROTI®Fect plus in 50 µl PBS each for 20 mins.
Transfection	within 1 hour after seeding in 0.5 ml full medium
Incubation	in medium 24 h (37 °C, 5 % CO_2)
Detection	after 24 and 48 h by fluorescence microscopy
Result	24 h post transfection: Best transfection efficiency (30 %) with ratio 1:4 (0.5 g / 2 µl ROTI®Fect plus) 48 h post transfection: Best transfection efficiency (20 %) with ratio 1:8 (0.5 g / 4 µl ROTI®Fect plus) (rate with ratio 1:4 was lowered to 22 %) Virtually no cell toxicity (< 2 %)

Cells / Cell line: SH-SY5Y Human Neuroblastoma

A)

Seeding	12wells, 2x10^5 cells / well, prior to transfection, incubate overnight -> 80-90 % confluency.
Medium	EMEM incl. 10 % FBS + antibiotics (amphot., pen. strept.) / OPTIMEM
DNA / RNA	pEGFP construct
Transfection complex	Dilute ROTI®Fect plus with 100 µl OPTIMEM, incubate for 15 mins at RT. Then add DNA as follows: ROTI®Fect plus : DNA in ratios of (µl : µg) 1:0.5, 2:0.5, 4:0.5, 6:0.5, 2:1, 4:1, 8:1, 12:1, 4:1.5, 8:1.5, 12:1.5 or 16:1.5 incubate mix at RT for 15 mins.
Transfection	Wash cells 2x with OPTIMEM, overlay with 1 ml pure OPTIMEM. Add transfection complex and mix gently.
Incubation	Incubate for 24 hours.
Detection	Wash cells with PBS and assay by confocal fluorescence microscopy.
Result	Most ratios showed transfection. Best results (80-98 % transfection efficiency) were obtained with ratios of: 6:0.5, 4:1, 12:1 and 12:1.5

Cells / Cell line: SW480 (Human Colon Carcinoma)

A)

Cell density	12wells, 2x10^5 cells / ml, 24 h prior to transfection
Medium	x
DNA / RNA	Plasmid with GFP
Transfection complex	1,1 µg Plasmid-DNA + 50 µl serum free medium + 3 µl ROTI®Fect plus in serum free medium Formation of transfection complex for 20 mins. at ambient temp.
Transfection	in 1 ml medium + FCS over night
Incubation	in full medium (37 °C, 5 % CO_2) for 48 hours overall
Detection	FACS and fluorescence microscopy
Result	ROTI®Fect plus is well tolerated by the cell line with very low to no toxicity. (Fugene HD, used in parallel with the same parameters, did show significant toxicity.) Transfection rate was 17 %. (Fugene HD: 9,4 %)

Cells / Cell line: Vero (Green Monkey kidney)

A) Reverse Transfection Cell Microarray (RTCM) on glass slides

Seeding	384well plates, 2,15x10^4 cells / cm^2, seeded after application of the transfection complex, confluency after 48 h
Medium	DMEM incl. 10 % FCS + PenStrep
DNA / RNA	pGFP
Transfection complex	ROTI®Fect plus 2, 3, 4 µl DNA 1, 2, 3, 4 µg mix DNA and ROTI®Fect plus add 3 µl serum-/antibiotics-free OPTI-MEM incl. 0.4 M sucrose add water to final vol. of 11.5 µl incubate for 20 mins.
Transfection	Mix transfection complex sol. with 7.25 µl 0.2 % gelatine and transfer to 384well plate with cells. Incubation time unknown.
Incubation	For 48 h in medium
Detection	Laser scan
Result	Transfection efficiency with ROTI®Fect plus was only 10 % in average. Results of two independent test series very inconsistent. Results also as inconsistent for Lipofectamine as they were found for ROTI®Fect plus. (Since this inconsistency was found for all assay performed in this study independent of ratio DNA/transfection agent or transfectant used, this may rather be a user's fault. Results are questionable.)

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