Technical Info

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Transfection Protocols ROTI®Fect – P001

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Cells / Cell line: A293 (Human transformed kidney)

A)

Cell density	12wells, 8x10^4 cells / ml, 50 % confluency after one night
Medium	x
DNA / RNA	Plasmid with LacZ
Transfection complex	1.5 µg Plasmid-DNA in 30 µl medium (ser^-/$antibiotics^-$) + 2.5 / 5 / 7.5 / 10 µl ROTI®Fect in 30 µl medium (ser^-/$antibiotics^-$) Formation of transfection complex for 15 mins. (RT)
Transfection	in 400 µl full medium for 5 hrs. (37 °C, 5 % CO_2)
Incubation	in 1 ml full medium (37 °C, 5 % CO_2)
Detection	X-gal staining of fixed cells
Result	Very low toxicity (lower than found for other transfection reagents). Very good transfection rates with ROTI®Fect of over 85 % in approaches with 5 µl ROTI®Fect and more. Best rate obtained with 10 µl ROTI®Fect (approx. 90 %).

Cells / Cell line: A431 (Epidermal Carcinoma)

A)

Cell density	12wells, 8x10^4 cells / ml, 50 % confluency after one night
Medium	x
DNA / RNA	Plasmid with LacZ
Transfection complex	1.5 µg Plasmid-DNA in 30 µl medium (ser^-/$antibiotics^-$) + 2.5 / 5 / 7.5 / 10 µl ROTI®Fect in 30 µl medium (ser^-/$antibiotics^-$) Formation of transfection complex for 15 mins. (RT)
Transfection	in 400 µl full medium for 5 hrs. (37 °C, 5 % CO_2)
Incubation	in 1 ml full medium (37 °C, 5 % CO_2)
Detection	X-gal staining of fixed cells
Result	Very low toxicity (lower than found for other transfection reagents). Low transfection rates with all reagents used (below 20 %). Best rate obtained with 10 µl ROTI®Fect (17 %). Use of ROTI®Fect plus (Art. No. CL21) recommended.

Cells / Cell line: BHK (Baby hamster kidney)

A)

Cell density	12wells, 8x10^4 cells / ml, 50 % confluency after one night
Medium	x
DNA / RNA	Plasmid with LacZ
Transfection complex	1.5 µg Plasmid-DNA in 30 µl medium (ser$^-$/antibiotics$^-$) + 2.5 / 5 / 7.5 / 10 µl ROTI®Fect in 30 µl medium (ser$^-$/antibiotics$^-$) Formation of transfection complex for 15 mins. (RT)
Transfection	in 400 µl full medium for 5 hrs. (37 °C, 5 % CO_2)
Incubation	in 1 ml full medium (37 °C, 5 % CO_2)
Detection	X-gal staining of fixed cells
Result	Very low toxicity (lower than found for other transfection reagents). Very good transfection rates with ROTI®Fect of 60 % in minimum in all approaches tried. Best rate obtained with 7.5 µl ROTI®Fect (approx. 95 %). 10 µl transfectant was slightly toxic.

Cells / Cell line: CHO (Chinese hamster ovary)

A)

Cell density	12wells, 8x10^4 cells / ml, 50 % confluency after one night
Medium	x
DNA / RNA	Plasmid with LacZ
Transfection complex	1.5 µg Plasmid-DNA in 30 µl medium (ser^-/$antibiotics^-$) + 2.5 / 5 / 7.5 / 10 µl ROTI®Fect in 30 µl medium (ser^-/$antibiotics^-$) Formation of transfection complex for 15 mins. (RT)
Transfection	in 400 µl full medium for 5 hrs. (37 °C, 5 % CO_2)
Incubation	in 1 ml full medium (37 °C, 5 % CO_2)
Detection	X-gal staining of fixed cells
Result	Very low toxicity (lower than found for other transfection reagents). Very good transfection rates with ROTI®Fect of over 65 % in all approaches tried. Best rate obtained with 10 µl ROTI®Fect (over 90 %).

Cells / Cell line: COLO205 (Human Colon Carcinoma)

A)

Cell density	6wells, 1.5x10^5 cells / ml
Medium	x
DNA / RNA	Plasmid with GFP
Transfection complex	2,5 µg Plasmid-DNA + 5 µl ROTI®Fect in serum / antibiotics-free medium Formation of transfection complex for 20 mins.
Transfection	approx. 4 hrs
Incubation	in full medium (37 °C, 5 % CO_2)
Detection	Fluorescence microscopy
Result	Very low transfection rate of approx. 2 %. Optimisation possible (DNA/lipid ratio). Use of ROTI®Fect plus (Art. No. CL21) recommended !

B)

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 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 2.7 %. Opinion is that this rate could be much higher if protocol would be optimised.

Cells / Cell line: COS1 (monkey kidney tissue)

A)

Cell density	24 wells, 8x 10^4 cells /well, incubation for several hours to let cells settle
Medium	DMEM and 10% FCS without antibiotics
DNA / RNA	pCMV (ß-Gal)
Transfection complex	0.15 or 0.32 or 1 µg Plasmid-DNA in 100 ml serum- free and antibiotic-free DMEM and 0.5 or 1.6 or 3 or 7 µl ROTI®Fect in serum- free and antibiotic-free DMEM, Mix and incubate for 20 min at RT
Transfection	5 hrs in serum-free DMEM, followed by a) or no incubation in serum free DMEM but b)
Incubation	a) 60 hrs in DMEM and 10% FCS, b) 65 hrs DMEM and 10% FCS both: 37 °C, 5 % CO_2
Detection	ß-Gal
Result	A relatively broad transfection optimum. The highest transfection efficiency was about 50 %. The 50 % efficiency was achieved with 1.6 µl ROTI®Fect and 1 µg or 0.32 µg DNA and incubating for 65 h in the presence of FCS. An almost equally high efficiency was achieved using 1 µg DNA and 0.5 µl ROTI®Fect and incubating for 65 h in the presence of FCS.

Cells / Cell line: COS7

A) RNAi mediated silencing of GFP synthesis

Cell density	6wells, ? cells / ml, 90-100 % optical confluency, ca. 70 %viability
Medium	DMEM + 10 % FBS + 1 % PenStrep
DNA / RNA	Expression plasmid pEGFP or -RFP
Transfection complex	2 µg Plasmid-DNA + 2 µl siRNA in 100 µl medium ser-free/antib.-free 10 µl ROTI®Fect in 100 µl medium ser-free/antib.-free combine and incubate for 15-20 mins.
Transfection	in fresh medium antib.-free, but serum containing, 2 ml / well add transfection complex and incubate
Incubation	in medium 24 h (37 °C, 5 % CO_2)
Detection	Fluorescence microscope
Result	Successful transfection, significant inhibition of GFP/RFP expression. Low cell toxicity.

B)

Seeding	12wells, ? cells / well, 24 hrs prior to transfection, confluency 90 %
Medium	DMEM incl. 10 % FBS + antibiotics
DNA / RNA	Expression plasmid pEGFP
Transfection complex	0.5 / 1 / 1.5 / 5 / 10 µg Plasmid-DNA in 100 µl medium ser-free/antib.-free 1 / 2 / 3 / 4 / 5 / 6 µl ROTI®Fect in 100 µl medium ser-free/antib.-free combine and incubate for 15-20 mins.
Transfection	a) for 4 hours, then replace with fresh medium, b) over night
Incubation	in full DMEM 24 hrs
Detection	Fluorescence microscope
Result	Best results found with 2,3, or 4, µl ROTI®Fect + 0.5 µg DNA 4, or 5, µl ROTI®Fect + 1 µg DNA 5, or 6, µl ROTI®Fect + 1.5 µg DNA up to 80 % transfection efficiency. Limiting factor = ratio ROTI®Fect : DNA Cells have to be in a high proliferative stadium, of about 90 % optical confluency. No toxic effects, even after incubation in transfection complex for 24 hours.

C)

Seeding	6wells, approx. 2 x 10^5 cells / well, 24 hrs prior to transfection, confluency ? %
Medium	Optimem incl. 10 % FBS + antibiotics
DNA / RNA	Expression plasmid pEGFP
Transfection complex	2 µg Plasmid-DNA in 100 µl medium ser-free/antib.-free 5 / 6 / 8 / 10 / 12 / 14 µl ROTI®Fect in 100 µl medium ser-free/antib.-free combine and incubate for 15-20 mins.
Transfection	in 2 ml medium, over night
Incubation	in full Optimem 48 hrs
Detection	Fluorescence microscope
Result	Best results found with 12 µl ROTI®Fect + 2 µg DNA (ratio of 6:1) In further approaches was found that indeed the ratio is vital, while the exact amounts are not that important. Keeping the ratio at 6:1 → very reproducible results (up to 75 % efficiency). Only a limited toxic effect was observed.

Cells / Cell line: HCT15 (Human Colon Carcinoma)

A)

Cell density	6wells, 1.5x10^5 cells / ml
Medium	x
DNA / RNA	Plasmid with GFP
Transfection complex	2,5 µg Plasmid-DNA + 5 µl ROTI®Fect in serum / antibiotics-free medium Formation of transfection complex for 20 mins.
Transfection	approx. 4 hrs
Incubation	in full medium (37 °C, 5 % CO_2)
Detection	Fluorescence microscopy
Result	Low transfection rate of approx. 10 %. Optimisation possible (DNA/lipid ratio). Use of ROTI®Fect plus (Art. No. CL21) recommended.

Cells / Cell line: HCT116 (Human Colon Carcinoma)

A)

Cell density	6wells, 1.5x10^5 cells / ml
Medium	x
DNA / RNA	Plasmid with LacZ reporter Gene
Transfection complex	2,5 / 5 µg Plasmid-DNA + 5 / 10 / 20 µl ROTI®Fect in serum / antibiotics-free medium Formation of transfection complex for 20 mins.
Transfection	approx. 4 hrs
Incubation	in full medium (37 °C, 5 % CO_2)
Detection	β-Gal Staining of fixed cells
Result	Best results when using 2.5 µg plasmid DNA + 5 µl ROTI®Fect reagent.

B)

Cell density	6wells, 1.5x10^5 cells / ml
Medium	x
DNA / RNA	Plasmid with GFP
Transfection complex	2,5 µg Plasmid-DNA + 5 µl ROTI®Fect in serum / antibiotics-free medium Formation of transfection complex for 20 mins.
Transfection	approx. 4 hrs
Incubation	in full medium (37 °C, 5 % CO_2)
Detection	Fluorescence microscopy
Result	Very good transfection rate of nearly 70 %, low toxicity.

C)

Cell density	12wells, 2x10^5 cells / well, 24 hours prior to transfection
Medium	x
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.

Cells / Cell line: HEK293

A) Cells in suspension

Cell density	24wells, 2x10^5 cells / ml, 80-100 % viability
Medium	HEKTOR-G protein-free medium, w/o FCS, with 2 mM L-Glutamine
DNA / RNA	GFP-plasmid construct
Transfection complex	1.5 µl Plasmid-DNA (conc. unknown) + 3.5 µl ROTI®Fect in 50 µl medium 15-20 min: addition of cells to mentioned density
Transfection	8 hrs
Incubation	in medium 18-72 h (37 °C, 5 % CO_2)
Detection	Microscope
Result	Transfection rate in suspended cells less than in adherent HEK293, but good. Medium is the important factor! Other media didn't work.

B)

Seeding	12wells, 1.5x10^5 cells / well, 24 hrs prior to transfection
Medium	DMEM incl. 10 % FBS + antibiotics
DNA / RNA	Luc-plasmid construct
Transfection complex	ROTI®Fect 1 – 16 µl : DNA 0.5 / 1 / 1.5 µg in serum-/antiobiotics-free medium
Transfection	18 hrs.
Incubation	in DMEM incl. 10 % FBS, 48 hrs
Detection	Luciferase assay system (RLU) and cell viability
Result	best results with ROTI®Fect : DNA 2 µl : 0.5 µg or 1 µl :0.5 µg or 2 µl : 1 µg.

C)

Seeding	6wells, 5x10^5 cells / well, 24 hrs prior to transfection, density 90 %
Medium	???
DNA / RNA	GFP-plasmid construct
Transfection complex	ROTI®Fect 5 µl : DNA 1 µg, preincubation for 15 mins in 50 µl medium w/o antib./serum;
Transfection	transfection in serum-/antiobiotics-free medium over night
Incubation	16 h
Detection	Microscope
Result	Better results with ROTI®Fect than with Reagents "F" and "L"

D)

Seeding	10 cm petri dishes, subconfluently, 24 hrs prior to transfection
Medium	DMEM incl. 5 % FBS + antibiotics
DNA / RNA	GFP-plasmid construct
Transfection complex	ROTI®Fect 20/30/40 µl : DNA 10 µg preincubation for 20 mins in 170 µl medium w/o antib./serum;
Transfection	In 4 ml medium, 24 hrs.
Incubation	-
Detection	Microscope
Result	In all tests over 75 % transfection efficiency. Slightliy best results with 40 µl.

Cells / Cell line: HeLa (Human cervix carcinoma)

A)

Seeding	12wells, 2x10^5 cells / well, 24 hrs prior to transfection, → ca. 80 % confluency
Medium	DMEM incl. 10 % FCS plus antibiotics over night, then 6h incubation in serum free DMEM
DNA / RNA	luciferase-test plasmid with CMV promoter
Transfection complex	ROTI®-Fect: 2.5 / 5 / 10 µl DNA: 0.5 / 1 / 2 µg, ratio DNA : ROTI®Fect 1:5 incubation for 20 mins (complex formation)
Transfection	transf. in 800 µl serum-containing, antiobiotics-free DMEM medium for 90 mins.
Incubation	wash in PBS, then incubate in DMEM plus serum (10 %) for 7 h
Detection	luciferase reporter assay
Result	All approaches with ROTI®Fect gave very good results, DNA amount was not very significant. Ratio seems to be significant. Cells healthy and stable. Results much better than those obtained with Dendrimer S.

B)

Seeding	12wells with cover slips, ? cells / well, 24 hrs prior to transfection, → ca. 90 % optical confluency
Medium	DMEM incl. 10 % FCS plus antibiotics over night
DNA / RNA	EGFP-tagged plasmid DNA
Transfection complex	ROTI®-Fect: 2 / 4 / 5 µl in 50 µl DMEM ser-free DNA: 0.5 / 1.5 µg in 50 µl DMEM ser-free various ratios DNA : ROTI®-Fect incubation for 20 mins (complex formation)
Transfection	transf. in 1 ml serum-containing DMEM medium for 4 h or over night.
Incubation	in DMEM plus serum (10 %) (either after removal of the transfection mix or including the transfection mix.) for 24 h. Then wash in PBS and fix cells.
Detection	Fluorescence microscopy
Result	Very good results (over 90 % transfection rate) with the following approaches: 4 µl ROTI®Fect / 1 µl DNA for 4 h 5 µl ROTI®Fect / 1.5 µl DNA for 4 h 4 µl ROTI®Fect / 1 µl DNA over night 5 µl ROTI®Fect / 1.5 µl DNA over night The worst results (<10 %) were obtained with a ration of 3:4 (1.5 µg DNA / 2 µl ROTI®-Fect). Very little toxic effects!

C)

Seeding	10 cm petri dishes, subconfluently, 24 hrs prior to transfection
Medium	DMEM incl. 5 % FBS + antibiotics
DNA / RNA	GFP-plasmid construct
Transfection complex	ROTI®Fect 20/30/40 µl : DNA 10 µg preincubation for 20 mins in 170 µl medium w/o antib./serum;
Transfection	In 4 ml medium, 24 hrs.
Incubation	-
Detection	Microscope
Result	In all tests over 75 % transfection efficiency. Slightliy best results with 40 µl.

Cells / Cell line: HeLa S3 (Human cervical carcinoma)

A)

Cell density	24 wells, 8x 10^4 cells /well, incubation for several hours to let cells settle
Medium	DMEM and 10% FCS without antibiotics
DNA / RNA	pCMV (ß-Gal)
Transfection complex	0.15 or 0.32 or 1 µg Plasmid-DNA in 100 ml serum- free and antibiotic-free DMEM and 0.5 or 1.6 or 3 or 7 µl ROTI®Fect in serum- free and antibiotic-free DMEM, Mix and incubate for 20 min at RT
Transfection	5 hrs in serum-free DMEM, followed by a) or no incubation in serum free DMEM but b)
Incubation	a) 60 hrs in DMEM and 10% FCS, b) 65 hrs DMEM and 10% FCS both: 37 °C, 5 % CO_2
Detection	ß-Gal
Result	Highest transfection efficiency (45%) using 1 µg DNA and 7 µl ROTI®Fect and incubating for 65 h in the presence of FCS. Transfection efficiency was about 29% using 1µg DNA and 1.6 µl ROTI®Fect and transfecting for 5h without FCS.

Cells / Cell line: Hep3B (Human hepatocellular carcinoma)

A)

Cell density	24 wells, 8x 10^4 cells /well, incubation for several hours to let cells settle
Medium	DMEM and 10% FCS without antibiotics
DNA / RNA	pCMV (ß-Gal)
Transfection complex	0.15 or 0.32 or 1 µg Plasmid-DNA in 100 ml serum- free and antibiotic-free DMEM and 0.5 or 1.6 or 3 or 7 µl ROTI®Fect in serum- free and antibiotic-free DMEM, Mix and incubate for 20 min at RT
Transfection	5 hrs in serum-free DMEM, followed by a) or no incubation in serum free DMEM but b)
Incubation	a) 60 hrs in DMEM and 10% FCS, b) 65 hrs DMEM and 10% FCS both: 37 °C, 5 % CO_2
Detection	ß-Gal
Result	Highest transfection efficiency using 1.6 or 3 µl ROTI®Fect and 1 µg DNA. Incubation with the ROTI®Fect/DNA complexes under serum free conditions resulted in *slightly* higher transfection rates (4 to 5 %)

B)

Seeding	10 cm culture dish, 1 x 10^6 cells /well, inoculated into fresh medium and let grow exponentially at the time of transfection.
Medium	DMEM and 10% FCS without antibiotics
DNA / RNA	pCMV (ß-Gal´)
Transfection complex	50 µl ROTI®-Fect, 10 g DNA in 600 µl medium each, without FCS or antibiotics, mix and incubate for 30 min at RT
Transfection	6 hrs in medium without FCS or antibiotics
Incubation	In DMEM incl. 10 % FCS and antibiotics, 72 hrs
Detection	ß-Gal
Result	40% transfection efficiency.

Cells / Cell line: HepG2 (Human hepatocellular carcinoma)

A) comparison of non-liposomal lipid, activated dendrimer and ROTI®Fect

Cell density	6 wells, ca. 2x 10^5 cells / well, ca. 70 % confluency
Medium	RPMI incl. 10% FCS, penicillin, streptomycin
DNA / RNA	Plasmid. pSV ß-Gal
Transfection complex	2 µg Plasmid-DNA + 10 µl ROTI®Fect in 100 µl medium without serum, without antibiotics. Mix and incubate for ca. 30 min.
Transfection	6 hrs
Incubation	In medium 24-48 h (37 °C, 5 % CO_2)
Detection	Photometrically (ß-Gal assay)
Result	Transfection with ROTI®Fect resulted in much higher transfection efficiency than both other methods (dendrimers ca. 20%)

B)

Seeding	12 wells, 1,5 x 10^5 cells / well
Medium	RPMI incl.10% % FBS + antibiotics
DNA / RNA	Plasmid
Transfection complex	ROTI®Fect 1 to 12 µl : DNA 0.5-1.5 µg in 100 µl serum-/antiobiotics-free medium each. Mix and incubate for 20 mins at RT.
Transfection	24 hrs in 1ml RPMI without serum, without antibiotics
Incubation	
Detection	Photometrically (ß-Gal assay)
Result	Best transfection efficiency:1.5 µg or 1.0 µg DNA and 4 µl ROTI®Fect. Very good efficiency: 1.0 µg DNA and 2 µl ROTI®Fect, and 1.5 µg DNA and 8 µl ROTI®Fect. 0.5 µg DNA and 1 µl ROTI®Fect was not enough for high level transfection. 12 µl ROTI®Fect also resulted in a much lower transfection efficiency (40-50%)

Cells / Cell line: HT22 (mouse hippocampal)

A)

Cell density	24wells, may be necessary to coat with gelatine. 3 x 10^4 cells / well in 800 µl DMEM, incubation ca. 1 day. Do not overgrow! Cells have to be exponentially growing!
Medium	DMEM + FCS (10 %) + Antibiotics
DNA / RNA	Plasmid, recombinant, GFP
Transfection complex	ROTI®-Fect: 3.0 µl / 3.0 µl / 4.0 µl in 50 µl DMEM w/o ser./antibiotics DNA: 0.8 µg / 1.0 µg / 1.3 µg in 30 µl DMEM w/o ser./antibiotics mix by pipetting incubate for 20 mins at RT
Transfection	add to cells plus medium
Incubation	4 hrs, then change medium. Whole: 40 hrs.
Detection	Fluorometric read out
Result	Optimal DNA / ROTI®Fect ratio is 1.3 µg : 4.0 µl (60-70 %). Low cell toxicity.

Cells / Cell line: HT29 (Human Colon Carcinoma)

A)

Cell density	12wells, 8x10^4 cells / ml, 50 % confluency after one night
Medium	x
DNA / RNA	Plasmid with LacZ
Transfection complex	1.5 µg Plasmid-DNA in 30 µl medium (ser$^-$/antibiotics$^-$) + 2.5 / 5 / 7.5 / 10 µl ROTI®Fect in 30 µl medium (ser$^-$/antibiotics$^-$) Formation of transfection complex for 15 mins. (RT)
Transfection	in 400 µl full medium for 5 hrs. (37 °C, 5 % CO_2)
Incubation	in 1 ml full medium (37 °C, 5 % CO_2)
Detection	X-gal staining of fixed cells
Result	Very low toxicity (lower than found for other transfection reagents). Very low transfection rates with all reagents used (below 1%). Best rate obtained with 10 µl ROTI®-Fect. Use of ROTI®Fect plus (Art. No. CL21) recommended.

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

A) antisense oligonucleotide transfection

Cell density	gelatine-coated 6wells, ? cells / well, 90-95 % optical confluency (ca. 4-5 days)
Medium	M199 plus 10 % FCS, antibiotics, HEPES, TES, heparine, EGF
DNA / RNA	antisense oligonucleotide
Transfection complex	5 µg antisense oligo in 100 µl M199 (ser- and antibiot.-free) 10 µl ROTI®Fect in 100 µl M199 (ser- and antibiot.-free) Mix and incubate 20 mins. at RT
Transfection	in Optimem 1 medium (2 ml / well) 3.5 hrs
Incubation	erplace by fresh M199 (full) and incubate 24 h (37 °C, 5 % CO_2)
Detection	Western-Blot of the extracts, PVDF membrane, chemoluminscence assay
Result	Reduction of reporter protein by over 60 %.

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

A)

Cell density	6well culture plates, 1x10^6 cells / well (24 h)
Medium	RPMI-1640 plus 10 % FCS, antibiotics
DNA / RNA	GFP-reporter plasmid
Transfection complex	1 / 4 / 8 / 14 µl ROTI®Fect in 50 ml RPMI (ser-free) 2 µg DNA in 50 ml RPMI (ser-free) incubate 15 mins. at RT
Transfection	in 1 ml RPMI (w/o serum / antibiotics?) for 24 hrs.
Incubation	24 hrs.
Detection	flowcytometry
Result	Best transfection rate was obtained when using 2 µg DNA and 14 µl ROTI®Fect (ratio 1:7) (>65 %). Nearly as good was the approach with 2 µg DNA and 8 µl ROTI®Fect (1:4) (63.24 %). Cell viability was very high.

Cells / Cell line: LoVo (Human Colon Carcinoma)

A)

Cell density	6wells, 1.5x10^5 cells / ml
Medium	x
DNA / RNA	Plasmid with GFP
Transfection complex	2,5 µg Plasmid-DNA + 5 µl ROTI®Fect in serum / antibiotics-free medium Formation of transfection complex for 20 mins.
Transfection	approx. 4 hrs
Incubation	in full medium (37 °C, 5 % CO_2)
Detection	Fluorescence microscopy
Result	Transfection rate of approx. 20 %. Transfected cells are vital, low toxicity. Enhancement of rate expected under optimization of the ratio DNA/ROTI®-Fect. Use of ROTI®Fect plus (Art. No. CL21) may bring better 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 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 14.8 %.

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 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 0.2 %. Opinion is that this rate could be much higher if protocol would be optimised.

Cells / Cell line: NIH3T3

A)

Seeding	24wells, 2x10^4 cells / well, 24 hrs prior to transfection
Medium	DMEM incl. 5 % FBS
DNA / RNA	GFP-plasmid construct under CMV promotor
Transfection complex	1 µg DNA + 1 / 2 / 3 µl ROTI®Fect in serum-/antiobiotics-free medium after 15 min: addition of 350 µl DMEM (incl. 5 % FBS)
Transfection	3 hrs or over night
Incubation	in DMEM incl. 5 % FBS, 24 hrs
Detection	FACS
Result	best results with 3 µl ROTI®Fect - over 90 % tr. efficiency for both, 3hrs. or ON transfection

B)

Seeding	24wells, approx. 24 hrs prior to transfection. transfection at 80 % confluency
Medium	DMEM incl. 10 % FCS
DNA / RNA	plasmid
Transfection complex	0.5 µg DNA + 2.5 µl ROTI®Fect in 50 µl serum-/antiobiotics-free medium
Transfection	3 hrs.
Incubation	in DMEM incl. 10 % FCS, 24 hrs
Detection	after fixation
Result	35 % tr. efficiency in first approach without any optimization

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 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 4.0 %. (Fugene HD 2.0 %)

Cells / Cell line: LLC-PK1

A) Establishment of stably transfected lines

Seeding	6wells, ? cells / well, 24 hrs prior to transfection, → ca. 60-70 % optical confluency
Medium	DMEM incl. 10 % FCS w/o antibiotics over night
DNA / RNA	EGFP-tagged plasmid DNA
Transfection complex	ROTI®-Fect: 4 µl in 100 µl DMEM ser-free DNA: 1 µg in 100 µl DMEM ser-free incubation for 20 mins (complex formation)
Transfection	add to 2 ml fresh medium (incl. serum) on cells
Incubation	for 24 h. Then wash in PBS and fix cells.
Detection	Fluorescence microscopy
Result	Good transfection efficiency. However, for establishment of stably expressing cells, subcloning was necessary. Higher transfection efficiency than found for other transfection reagents. No significant toxic effects found!

Cells / Cell line: SW480 (Human Colon Carcinoma)

A)

Cell density	6wells, 1.5x10^5 cells / ml
Medium	x
DNA / RNA	Plasmid with GFP
Transfection complex	2,5 µg Plasmid-DNA + 5 µl ROTI®Fect in serum / antibiotics-free medium Formation of transfection complex for 20 mins.
Transfection	approx. 4 hrs
Incubation	in full medium (37 °C, 5 % CO_2)
Detection	Fluorescence microscopy
Result	Good transfection rate of approx. 35 %. Transfected cells are vital, low toxicity. Further enhancement of rate expected under optimization of the ratio DNA/ROTI®-Fect. Use of ROTI®Fect plus (Art. No. CL21) may bring better results.

B)

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 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 24.6 %. (Fugene HD: 9.4 %)

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