

Transfection Reagent: Roti[®]-Fect (P001)

Cells / Cell line: HeLa (Human cervix carcinoma)



Protocols and Results

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, antibiotics-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. Slightly best results with 40 µl.

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