

## Transfection Reagent: Roti<sup>®</sup>-Fect (P001)

Cells / Cell line: COS7

### Protocols and Results

#### 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 <sup>®</sup> -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 <sub>2</sub> )
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 <sup>®</sup> -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 <sup>®</sup> -Fect + 0.5 µg DNA 4, or 5, µl Roti <sup>®</sup> -Fect + 1 µg DNA 5, or 6, µl Roti <sup>®</sup> -Fect + 1.5 µg DNA up to 80 % transfection efficiency. Limiting factor = ratio Roti <sup>®</sup> -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 <sup>5</sup> 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 <sup>®</sup> -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 <sup>®</sup> -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.

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