

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

Cells / Cell line: HCT116 (Human Colon Carcinoma)

Protocols and Results

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.

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