

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

Cells / Cell line: COLO205 (Human Colon Carcinoma)

Protocols and Results

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	Rot [®] -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.

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