

Transfection Reagent: Roti[®]-Fect plus (CL21)

Cells / Cell line: HCT116 (Human colon carcinoma)

Protocols and Results

A)

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

B)

Cell density	6wells, 1,5x10 ⁵ cells / well, 48 hours prior to transfection
Medium	DMEM, 10 % FCS, Pen/Strep, HEPES
DNA / RNA	Plasmid with GFP
Transfection complex	1 µg DNA Plasmid-DNA in 50 µl serum/antib. free medium + 2, 4, 6 µl Roti [®] -Fect plus in 50 µl serum/antib. free medium Mix and incubate for formation of transfection complex for 15 mins.
Transfection	add transfection complex to the cells (with / w/o medium?). Incubate for 5 h.
Incubation	Change medium after 5 hours, incubate 24 h in full medium (37 °C, 5 % CO ₂)
Detection	Fluorescence microscopy
Result	Good transfection rate with 4 µl Roti [®] -Fect plus. Low toxicity. However, for other cell lines 2 or 6 µl Roti [®] -Fect plus gave better results. Amounts must be optimised for each cell line.

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