

Transfection Reagent: Roti®-Fect (P001)

Cells / Cell line: HEK293

Protocols and Results



A) Cells in suspension

Cell density	24wells, 2×10^5 cells / ml, 80-100 % viability
Medium	HEKTOR-G protein-free medium, w/o FCS, with 2 mM L-Glutamine
DNA / RNA	GFP-plasmid construct
Transfection complex	1.5 μ l Plasmid-DNA (conc. unknown) + 3.5 μ l Roti®-Fect in 50 μ l medium 15-20 min: addition of cells to mentioned density
Transfection	8 hrs
Incubation	in medium 18-72 h (37 °C, 5 % CO ₂)
Detection	Microscope
Result	Transfection rate in suspended cells less than in adherent HEK293, but good. Medium is the important factor! Other media didn't work.

B)

Seeding	12wells, 1.5×10^5 cells / well, 24 hrs prior to transfection
Medium	DMEM incl. 10 % FBS + antibiotics
DNA / RNA	Luc-plasmid construct
Transfection complex	Roti®-Fect 1 – 16 μ l : DNA 0.5 / 1 / 1.5 μ g in serum-/antibiotics-free medium
Transfection	18 hrs.
Incubation	in DMEM incl. 10 % FBS, 48 hrs
Detection	Luciferase assay system (RLU) and cell viability
Result	best results with Roti®-Fect : DNA 2 μ l : 0.5 μ g or 1 μ l : 0.5 μ g or 2 μ l : 1 μ g.

C)

Seeding	6wells, 5×10^5 cells / well, 24 hrs prior to transfection, density 90 %
Medium	???
DNA / RNA	GFP-plasmid construct
Transfection complex	Roti®-Fect 5 μ l : DNA 1 μ g, preincubation for 15 mins in 50 μ l medium w/o antib./serum;
Transfection	transfection in serum-/antibiotics-free medium over night
Incubation	16 h
Detection	Microscope
Result	Better results with Roti®-Fect than with Reagents "F" and "L"

D)

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 μ l : DNA 10 μ g preincubation for 20 mins in 170 μ l 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 μ l.

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