



Transfektionsprotokolle ROTI®Fect RNAi - 3129

Cells / Cell line: HeLa (Human cervix carcinoma)

A)

Seeding	6wells, 8×10^4 cells / well, 24 hrs prior to transfection
Medium	DMEM incl. 10 % FCS plus antibiotics overnight,
DNA / RNA	GTPase siRNA
Transfection complex	Buffer: 180 μ l ROTI®Fect RNAi: 12 μ l siRNA: 180 pmol incubation for 15 mins (complex formation)
Transfection	Wash cells with PBS, overlay with transfection complex. Add 1.8 ml full DMEM.
Incubation	24 up to 48 hours
Detection	SDS-PAGE -> Western-Blot
Result	Normalization of the Blot against tubulin. Silencing ca. 95 % after 48 h (approx. 35 % after 24 h). Similar silencing rates with Oligofectamin needed 5 days incubation.

B)

Seeding	24wells: 0.4×10^5 cells / well, and 6wells: 3.5×10^5 cells / well 24 hrs
Medium	DMEM incl. 10 % FCS plus antibiotics overnight,
DNA / RNA	siRNA, fluorescent plasmid (GFP) for evaluation of transfection efficiency
Transfection complex	Buffer: 60 μ l / 180 μ l ROTI®Fect RNAi: 4 μ l / 12 μ l siRNA: 60 pmol / 180 pmol incubation for 15 mins (complex formation)
Transfection	Overlay washed with transfection complex and add 500 μ l or 2 ml full DMEM.
Incubation	24 until fluorescence microscopy, 48 hours for silencing
Detection	fluorescence microscopy and SDS-PAGE -> Western-Blot
Result	No cytotoxicity detectable (Phase contrast microscopy), no increased apoptosis, no morphology changes. Silencing of 3.5×10^5 cells / 6well --> rate of less than 20 %. Silencing of 0.4×10^5 cells / 24well --> rate of less than 60-90 % and good fluorescent signals (marker plasmids). Results were comparable to that achieved with RNAiMax.



Technische Info



Guter Rat ist Roth.

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Die Firma ist eine Kommanditgesellschaft mit Sitz in Karlsruhe, Reg. Gericht Mannheim HRA 100055. Persönlich haftende Gesellschafterin ist die Roth Chemie GmbH mit Sitz in Karlsruhe, Reg. Gericht Mannheim HRB 100428. Geschäftsführer: André Houdelet

