



Transfection Protocols 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.



Well advised with Roth.

Technical Info

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