Instructions for use

ROTI®Fect RNAi Kit

Reagent for transfection of mammalian cells with siRNA and miRNA, for efficient gene silencing

Product Description
New, powerful transfection reagent for liposoma mediated siRNA and miRNA transfection of all mammalian cells in culture. Liposoma formulation and enclosed buffer were specially established and designed for core parameters of RNAi: Most efficient quantitative endocytosis plus release of nucleic acids into the cytosol, without subsequent transport to the nucleus.
ROTI®Fect RNAi provides you with superior knockdown results, even using low amounts of RNA. Hereby, the ROTI®Fect technology guarantees minimisation of toxic effects during transfection, thus making these reagents suitable for very sensitive cell lines of primary cells. The detailed instruction-for-use contains a standardised transfection protocol working via a definite RNA/liposoma ratio and providing good results starting with the very first assay - making elaborate optimisations of transfection obsolete. Evaluation of results is often possible after 48 hours, enabling two sequential assays per week.
One milliliter ROTI®Fect RNAi is sufficient for 650 transfections in 16 mm dishes (24wells). ROTI®Fect RNAi is provided as a ready-to-use solution plus an optimised RNAi transfection buffer.

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Stability:
ROTI®Fect RNAi Kit comes as a ready-to-use solution and can be stored at 4°C for an extended period of time.
For optimal results, it is recommended to subject the solution to a freeze-thaw cycle every 4 weeks. This ensures that the liposomes return to their original state, maintaining transfection efficiency.

Culture media:
recommended for RNAi transfection with whole medium

Shipping: At room temperature (freeze after receipt)

Only for research purposes *in vitro*, not intended for human or animal diagnostic, therapeutic, or other clinical uses.

Application notes:
RNA is sensitive to ubiquitous RNases. All reagents and vessels used must be RNAse-free.

Quality Control:
Performance and quality is evaluated in a standard transfection assay. Absence of bacterial and fungal contamination is verified using fluid thioglycolate medium.

Cells and Cell Lines Successfully Transfected with ROTI®Fect RNAi :
Primary cells: MCS, M1
Cell lines: A549, 3T3-L1, B16F10, CHO-K1, H441, HT1080, HeLa, HeLa-KB, HepG2

Working Instructions

I) Preliminary Notes

Condition of cells
The cells to be transfected should be in a healthy condition and in the proliferation phase (70-90 % confluency). We recommend using only regularly passaged cells for transfection experiments. Microbial contamination, for example with mycoplasma or fungi, must be avoided because this can falsify transfection results.
Quality of the genetic material
The RNA should be of the highest possible purity in order to achieve best knockdown results.
Purity of reagents and vessels used
RNA is sensitive to ubiquitous RNases. All reagents and vessels used must be RNAse-free.

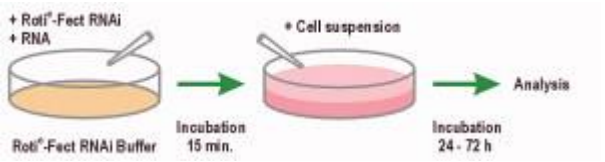
II) Protocol

A) Preparations
1. Calculate the required quantity of 1x RNAi buffer by multiplying the number of transfected wells by the buffer quantity specified in the table (p. 2). Dilute 1/ 10 vol. ROTI®Fect RNAi buffer (10x) with 9/10 vol. endotoxin and RNAse-free water. Mix by vortexing.
The 1x buffer can also be produced in larger quantities and frozen in aliquots.
2. Prior to the transfection procedure, 1x RNAi buffer, ROTI®Fect RNAi liposoma formulation and RNA solution are to be equilibrated to room temperature and briefly vortexed.
3. Make up 250 µl of cell suspension with a concentration of $0.8x10^5$ cells per ml in whole culture medium.

B) Making the RNA/lipo complex
These instructions refer to a well with 1 cm^2 of growth surface (48well). Refer to the table below for values for other well sizes. The specified quantity of RNA/lipo complex has been optimised and can be used for most formulations. For optimisation instructions, refer to (III).
1. Pipette 30 µl ROTI®Fect RNAi buffer (1x) into a well to produce a buffer droplet.
2. Pipette the following reagents into this droplet one after the other: 2 µl ROTI®Fect RNAi Lipo and 30 pmol RNA. Mix the contents by gently pipetting up and down.
3. Incubate at room temperature for 15 mins.

C) Transfection
Pipette 250 µl of the prepared cell suspension into the well containing the RNA lipo complex droplet. Incubate the cells without any further mixing for 24-72 hours under the usual conditions for cells or cell lines.

D) Evaluation
After 24-72 hours evaluate the formulation using the usual assay method (e.g. real-time PCR, reporter gen assay). The highest knockdown rate is usually achieved 48 hours after transfection. The optimal time for evaluation is dependent on the characteristics of the cell line, the expression rate of the protein to be deactivated and the half-life of the protein in the expressing cell.



III) Optimisation

A) Adjusting the complex quantity

If necessary, the complex quantity of RNA/lipo can be halved (e.g. in cases of highly sensitive cells) or doubled (e.g. to further increase the knockdown rate). To this end, both volumes – ROTI®Fect RNAi Lipo **and** RNA – have to be increased or reduced accordingly. The quantities of RNAi buffer (1x) and cell suspension are not adjusted.

B) Adjusting the number of cells

If the number of cells per well is increased/reduced, the complex quantity to be added must be increased/reduced proportionally.

The liposome formula to RNA ratio must be kept constant at all times:
1 µl ROTI®Fect RNAi Lipo for 15 pmol RNA

IV) Reagent Quantities for Culture Vessels of Different Size

culture Dish Ø (mm)	**6 (96well)**	**11 (48well)**	**16 (24well)**	**22 (12well)**	**35 (6well)**
Area per well (cm^2)	0.3	1	1.9	3.9	9.6
cell suspension (µl)	100	250	500	1000	2000
RNA amount (pmol)	15	30	60	120	180
ROTI®Fect RNAi Lipo-amount (µl)	1	2	4	8	12
ROTI®Fect RNAi buffer (1x) amount (µl)	15	30	60	120	180

Trouble Shooting

Possible reason	**Comments**
Low transfection efficiency	
Cells are not proliferating well or are not healthy	Cells, which have been sitting at confluency for a while may not transfect as efficiently as cells, which are growing rapidly. Therefore, it is recommended to use regularly passaged cells for transfection experiments.
Cell confluency too high	If cell density during transfection is too high, this might lead to insufficient uptake of the complex.
Poor RNA quality	RNA should be of highest purity for optimal knock-down results.
Wrong serum concentration	Knock-down should be performed in foll medium.
Duration of knock-down too short	Extension of knock-down duration to up to 72 hours is possible.
Cells too old	Use only freshly seeded and regularly passaged cells for transfection.
DNA and ROTI®Fect RNAi amount suboptimal	Use only ratio of 1 µl ROTI®Fect RNAi Lipo to 15 pmol RNA.
Excessiv cell death	
Amount of RNA/Lipo complex too high	Reduce.
Incubation time with RNA/Lipo complex too long	Reduce to 24 hours.
Cells are stressed	Avoid temperature shifts and long periods without medium during preparation of cell suspension. Perform transfection in full medium so that cells are not deprived of necessary growth factors and nutrients.
Low reproducibility	
Varying rates of cell confluency	Ensure constant cell number for all experiments (seed same cell number and maintain constant incubation times between seeding and complex addition).
Microbial contamination	Microbial contamination for example with mycoplasma or fungi can drastically alter transfection results, leading also to false-negative results, hence the lack of knock-downs.
Cells have beenpassaged too many times	Morphology and therewith transfection results can alter with extended number of passaging. Often transfection and knock-down efficiency decreases at high passaging numbers.

RNA Interference

RNA Interference (RNAi) is a natural regulatory mechanism in gene expression, controlled by short-chain RNA with approx. 21-28 nucleotides which are complementary to the mRNA of a target protein and can be found in all eukaryotic cells.
A distinction is made here between siRNA and miRNA, which is formed in the case of siRNA from synthetic double-strand RNA, and, in the case of miRNA, from endogenic hairpin-structure RNA by means of endoribonuclease cleavage (Dicer).
These small RNA strands are bound in the cytosol by a ribonucleoprotein complex (RISC complex = RNA induced silencing complex), separating the RNA double strand and presenting the leading strand. When a complementary mRNA strand binds to the leading strand, it is broken down or deactivated by the RISC complex, thus preventing expression of the target protein and down-regulating the corresponding gene (knockdown).
Unlike conventional transfection reagents for DNA transfection, reagents for the transfection of siRNA and miRNA do not need to transport the DNA into the cell nucleus. The development of ROTI®Fect RNAi was thus primarily aimed at ensuring the maximum endocytosis volume in the lipoplex plus rapid and complete release of RNA in the cytosol.

Recommended Reagents:

Water, sterile, pyrogen-free (1 Litre) – Ord. No. 3255.1
Water, DNAse/RNAse-free (50 x 1 ml) – Ord. No. T143.4
DEPC (25 ml) – Ord. No. K028.1

The set contains:

0.2 and 1 ml, respectively, ROTI®Fect RNAi liposoma formulation (Art. No. 2911, may not be bought separately), and 0.4 and 2 ml, respectively, ROTI®Fect RNAi buffer (Art. No. 2913). ROTI®Fect RNAi buffer may be bought separately.

ROTI®Fect RNAi Kit	**0,2 ml**	**3129.1**
	1,0 ml	**3129.2**