Instructions for use

Roti®-Fect

Reagent for the liposome-mediated transfection of eucaryotic cells

Product Description

Roti®-Fect is a liposome formulation of a polycationic lipid combined with a neutral colipid. It condenses DNA and RNA to compact structures (Roti®-Fect/nucleic acid-complexes) and ensures high efficient uptake into mammalien cells. Roti®-Fect shows no serum inhibition, which makes it a valuable tool for transfecting sensitive cell lines. Low toxicity results in successfull transfection of a broad cell line range.

Compared to monocationic lipid formulations (e.g. DOTAP) Roti®-Fect shows superior transfection results while less material is needed. Furthermore Roti®-Fect combines excellent reproducibility with a broad peak performance. Thus, no or only litte optimisation is necessary to yield high transfection efficiencies.

One milliliter Roti®-Fect is sufficient for 50-200 transfections in 35 mm dishes. Roti®-Fect is provided as a ready-to-use solution. It can be stored at 4 °C for more than a year without any loss of transfection efficiency. It was also shown to be stable for weeks at 40 °C.

Storage 4 °C
Shipping: At room temperature

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

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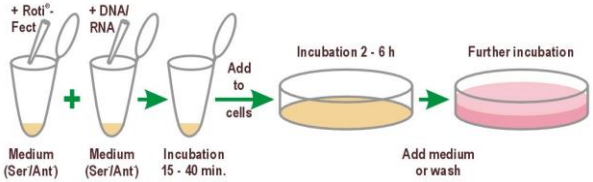
Important information:
Do not freeze, mix gently before use.

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

Cell Lines Successfully Transfected with Roti®-Fect :
143B, 293-T, A-293, A-431, AM-C6SC8, BHK, BV-2, C2C12, C6, CHO, CHO-DHFR, COS-1, COS-7, CRFK, Colo-205, DU-145, ECV-304, EMC, F98 (glioma), GD25-ß1, HBL 100, HCT-116, HCT-15, HEK293, HeLa, HeLa-S3, Hep-3B, Hep-G2, HOSE, HSG, HT-22, HT-29, HUVEC, JURKAT, LLC-PK1, LS174T, LoVo, MCF-7, MDCK, MeWo, MV3, NIH-3T3, NS20Y, OK, P815, PC3, PT-11, Rcho-1, SAOS, SH-SY5Y, SK-MEL-28, SKOV-3, SM10, SW-480, THP-1, tsA201, U87 (glioma), Vero 76, *et al.*

Working Instructions
Transfection of Adherent cells

1. Seed 1-3 x 10^5 cells in a 35 mm (6–well) dish in 3 ml of the appropriate complete growth medium.
2. Incubate the cells for 18-24 hours at 37 °C in a CO_2 incubator until they are app. 50-90 % confluent (The time required depends on cell type).
3. Vortex the stock solutions of DNA and transfection reagent gently prior to use. Both solutions should be brought up to room temperature.
4. Prepare the following solutions in tubes made of glass, polypropylen or polystyrene:
   A: 1-3 µg of DNA/RNA in 100 µl serum-free medium without antibiotics (we propose 2 µg in first experiment);
   B: 2.5-25 µl of Roti®-Fect transfection reagent in 100 µl serum-free medium without antibiotics(we propose 10 µl in first experiment);
   Mix the each solution gently by carefully pipetting several times.
   Note: Those two solutions have to be prepared freshly and used within 5 mins. Waiting periods reduce transfection efficiency.
5. a.) Combine the two solutions, mix gently by carefully pipetting several times, and allow the nucleic acid-lipid complex to form by incubating on bench top (at room temperature).This will require 15-40 min.
   b.) While complexes form rinse the cells once with PBS (phosphate buffered saline) and refill the dish with 1.5 ml fresh antibiotica-free medium with serum.
6. Add the nucleic acid-lipid complex to the cells, mix gently and incubate for 2-6 hours at 37 °C in a CO_2 incubator (we propose 6 h in first experiment).
7. If working with very sensitive cells the transfection medium should be removed completely and should be replaced by complete cell culture medium.
8. Incubate the cells with or without the complex for further 15-20 hours at 37 °C in a CO_2 incubator.
9. Replace the medium with fresh complete cell culture medium.
10. Depending on cell type and promotor activity, assay cell extracts for gene activity 24-72 hours following the start of transfection (we propose 24 h in first experiment).



Transfection of Non-adherent Cells

1. Wash the cells once with PBS (phosphate buffered saline).
2. Seed 2-3 x 10^6 cells in a 35 mm (6-well) dish in 1.5 ml fresh serum-free medium without antibiotics.
3. Vortex the stock solutions of DNA and transfection reagent gently prior use. Both solutions should be brought up to room temperature.
4. Prepare the following solutions using tubes made of glass, polypropylen or polystyrene:
   A: 1-3 µg of DNA/RNA in 100 µl serum-free medium and without antibiotics (we propose 2 µg in first experiment);
   B: 2.5-25 µl of Roti®-Fect transfection reagent in 100 µl serum-free medium without antibiotics (we propose 10 µl in first experiment);
   Mix each solution gently by carefully pipetting several times.
   Note: Those two solutions have to be prepared freshly and used within 5 mins. Waiting periods reduce transfection efficiency
5. Combine the two solutions, mix gently by carefully pipetting several times, and allow the nucleic-acid-lipid complex to form by incubating on bench top (at room temperature).This will require 15-40 min.
6. Add the nucleic-acid-lipid complex to the cell suspension, mix gently and incubate 2-6 h at 37 °C in a CO_2 incubator (we propose 6 h in first experiment).
7. Following incubation add 3 ml complete cell culture medium. If working with very sensitive cells, the transfection medium should be removed completely and should be replaced by complete cell culture medium.
8. Incubate the cells with or without the complex for further 18 hours at 37 °C in a CO_2 incubator.
9. Replace the medium with fresh complete cell culture medium.

10. Depending on cell type and promotor activity, collect cells by centrifugation and assay cell extracts for gene activity 24-72 hours following the start of transfection (we propose 24 h in first experiment).

Optimisation

Every combination of cell line and DNA/RNA has to be optimised in transfection efficiency with respect to transfection parameters**. Fortunately, Roti®-Fect performs a broad peak behavier. Thus, no or only little optimisation is necessary to yield satisfactory results.** As a rule, the optimisation of the ratio of Roti®-Fect to DNA/RNA as most important factor is sufficient.

Parameters, which Influence Transfection Results are:

1. Ratio of Roti®-Fect to DNA/RNA
2. Amount of transfection complex
3. Cell confluency
4. Serum concentration
5. Incubation time with transfection complex
6. Time range between transfection and reporter gene assay

Optimisation Strategy

Parameters can not be optimised independently step by step. The optimal ratio of Roti®-Fect/nucleic acid and the optimal amount of transfection complex, which are the most important parameters, depend on cell number to be transfected and serum concentration. Therefore, it is necessary to keep constant number of seeded cells and incubation period before transfection procedure (determines number of cells to be transfected) and the remaining conditions at the proposed starting points. DNA/RNA amount and Roti®-Fect amount are varied within the proposed intervals.

Example:

Seed a defined number of cells in three 6 well plates. Follow the working instruction and apply different DNA/RNA amounts for each 6 well plate (eg. 1, 1.5 and 2 µg DNA/RNA). Within one 6 well plate vary the amount of Roti®-Fect within the proposed range (e.g. 2, 4, 6, 8, 10, 12 µl Roti®-Fect). During incubation with the transfection-complex the serum concentration should be the same as cells are normally cultured with. Determine the optimal amounts of DNA/RNA and Roti®-Fect by reporter gene assays.

If results are to be optimised further change single parameters like cell number or serum concentration, and repeat determination of optimal amounts of DNA/RNA and Roti®-Fect.

After optimal cell number, serum concentration, DNA/RNA and Roti®-Fect amount are found, vary incubation time with transfection complex and time range between transfection and reporter gene assay.

Reagent Quantities for Different Size Culture Vessels

For first experiments we propose the values in brackets.

culture Dish Ø (mm)	**11 (48 well)**	**16 (24 well)**	**22 (12 well)**	**35 (6 well)**	**60**	**100**
Area per well (cm^2)	1	1.9	3.9	9.6	28.3	78,5
seeded cell number (x 10^5)	0.2-0.4	0.3-0.6	0.8-1.3	1-3	5-10	15-25
culture medium without antibiotics (ml) (step 5b)	0.16	0.25	0.5	1.5	3	9
DNA/RNA amount (µg)	0.1-0.5 (0.3)	0.2-1 (0.5)	0.5-2 (1)	1-4 (2)	2-10 (5)	5-20 (10)
Roti®-Fect amount (µl)	0.3-5 (1.5)	0.5-8 (2.5)	1-15 (5)	2.5-25 (10)	4-75 (25)	10-200 (50)
Dilution vol. (µl) of RNA and Roti®-Fect (step 4)	20	30	50	100	300	600
Vol. of transfection medium vol. (ml) (step 4)	0.2	0.31	0.6	1.7	3.6	10.2

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 DNA quality	DNA should be of highest purity, if optimal results for transfection is desired.
Presence of antibiotics during complex formation or incubation time	We do not propose transfection with Roti®-Fect in presence of antibiotics. Reports in literature and own results show, that in some cases presence of antibiotics reduce transfection efficiency.
DNA/RNA for too long in serum-free medium prior to transfection	Endtoxines considerably reduce transfection efficiency. Nucleic acids should not be incubated for more than 5 min in serum-free culture medium prior to transfection
Duration of transfection too short	With insensitive cells, extension of transfection duration to up to 2 days is possible.
Cells too old	Using freshly seeded cells can result in higher transfection efficiency.
Wrong serum concentration	Transfection can be performed under serum-reduced conditions.
DNA and Roti®-Fect amount suboptimal	Although Roti®-Fect shows a broad peak performance we recommend optimisation of the transfection protocol for each combination of plasmid and cell line used for optimal results. An instruction is given in the section optimisation.
Excessiv cell death	
Amount of nucleic acid/Roti®-Fect - complexes too high	Reduce.
Incubation time with nucleic acid /Roti®-Fect-complexes too long	Reduce.
Cells are stressed	Avoid temperature shifts and long periods without medium during washing steps. Perform transfection in presence of serum, so that cells are not deprived of necessary growth factors and nutrients.
Serum concentration too low or incubation time in serum-reduced culture medium too long.	Keep the serum concentration at levels usually used for culturing of this cell type.
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.
Cells have beenpassaged too many times	Morphology and therewith transfection results can alter with extended number of passaging. Often transfection efficiencies decrease at high passaging numbers.
Serum variability	Variations in serum quality can lead to variation in transfection efficiencies. Ensure same quality for all experiments.

Literature:

- Hennemann et al. (2003) *J Biol Chem* 278:28799-28811
- Ufartes et al., (2005) *BIOspektrum* 4:464-465
- Vassen et al. (2005) *Nucl Acids Res* 33:987-998

Roti®-Fect	**0.2 ml**	**P001.1**
	0.5 ml	**P001.2**
	1.0 ml	**P001.3**
	5 x 1.0 ml	**P001.4**