

Introduction

LiFectRNA™ Transfection Reagent is an innovative cationic nanotechnology developed specifically for the delivery of siRNA and miRNA into a wide range of eukaryotic cells. LiFectRNA™ Transfection Reagent provides the highest transfection efficiencies on the widest variety of cell types for siRNA mediated gene knockdown experiments. The reagent is compatible with the use of serum and antibiotics in the cell culture medium.

Package Information

Components	M0146-01	M0146-03
LiFectRNA™ Reagents	1 ml	3 ml

Protocols

Cell seeding

Cell seeding has to be adjusted according to your cell culture vessel. For optimal transfection conditions with LiFectRNA™, we recommend seeding the cells the day before transfection to reach 60-80% confluent cells on the day of transfection.

Table 2. Complexation parameters for the transfection of adherent cells.

Culture vessel	96-well	24-well	6-well
Adherent cells	1-4 × 10 ⁴	0.5-2 × 10 ⁵	0.25-1 × 10 ⁶

Preparation of the complexes

The recommended complexation parameters are described in Table 2.

Table 2. Complexation parameters for the transfection of adherent cells.

Parameter	96-well	24-well	6-well
siRNA	1 pmol	5 pmol	25 pmol
LiFectRNA™	0.4 µl	2 µl	8 µl

Transfection

The following protocol is given for transfection of adherent cells in a 24-well plate according to the recommended conditions in Table 2.

1. One day before transfection, plate 0.5-2 × 10⁵ cells in 500 µl of growth medium without antibiotics so that cells will be 60-80% confluent at the time of transfection.
2. Dilute 5 pmol of siRNA in 25 µl of Opti-MEM® Medium (or other medium without serum). Mix gently.
3. Mix LiFectRNA™ gently before use, then dilute 2 µl of LiFectRNA™ in 25 µl of Opti-MEM® Medium. Mix gently.
4. Add the 25 µl LiFectRNA™ solution onto the 25 µl siRNA solution all at once. Mix immediately the solution, either by briefly vortexing it or inverting the tube few times.
5. Incubate the complexes at room temperature for 15 minutes.
6. Add the 50 µl LiFectRNA™/siRNA mix to the cells in 500 µl of medium. Mix gently by rocking the plate back and forth.
7. Incubate cells at appropriate temperature and CO₂ levels (e.g. 37°C, 5%) for 1-3 days. Then, analyze transfected cells.

Optimizing gene silencing efficiency

To obtain the highest gene silencing efficiency, optimize transfection conditions by varying siRNA concentration from 10 nM to 50 nM. Then, adjust the corresponding amount of LiFectRNA™.