

PRODUCT INFORMATION

**Thermo Scientific
TurboFect siRNA Transfection
Reagent**

500 μ l (for 500 transfections in a 24-well plate)

#R1401

Lot: ___ Expiry Date: ___
Store at 4°C

www.thermoscientific.com/fermentas

Description

Thermo Scientific TurboFect siRNA Transfection Reagent is a sterile solution of a cationic polymer in water. The polymer forms compact, stable, positively charged complexes with siRNA and facilitates efficient siRNA delivery into the cytoplasm of cells.

The reagent delivers siRNA into a variety of cell types, including primary and suspension cells. It is also ideal for siRNA-DNA co-transfection. Transfection is highly efficient in the presence or absence of serum and antibiotics.

Reagents to be Supplied by the User
0.15 M NaCl, serum-free DMEM, RPMI or other serum-free medium.

General Considerations

Cell Density

The recommended confluency for adherent and suspension cells at the day of transfection is 70-90%.

Incubation Time

Incubate the transfection reagent / siRNA complexes with the cells until the gene silencing assay is performed (24-72 hours).

Transfection Reagent / siRNA Ratio

The volume of transfection reagent used depends on the amount of siRNA and type of cells to be transfected. The ratios presented in the protocols below are generalized values and can be further optimized for best results.

Transfection in the Presence of Serum and Antibiotics

Transfection reagent is compatible with serum and antibiotics.

General Protocol for siRNA Transfection of Adherent and Suspension Cells in a 24-well Plate

Quantities and volumes should be scaled-up according to the number of cells/well to be transfected (*see* Table 1).

Subsequent optimization of the quantities of siRNA and transfection reagent may further increase the transfection efficiency and result in more efficient gene silencing.

1. In a 24-well plate, seed $\sim 5 \times 10^4$ adherent cells or $\sim 1 \times 10^5$ suspension cells per well 24 h prior to transfection (use 0.5 ml of growth medium).

Note

The recommended confluency for adherent and suspension cells on the day of transfection is 70-90 %. *See* Table 1 for recommended number of cells to seed a day before transfection.

2. Dilute 3 pmol of siRNA in 100 μ l of 0.15 M NaCl or serum-free medium for a final siRNA concentration of 5 nM in the cell culture.
3. Briefly vortex TurboFect™ reagent and add 1 μ l of it to the diluted siRNA. Mix immediately by pipetting or vortexing.
Note
TurboFect/siRNA complexes should be prepared immediately prior to transfection
4. Incubate 15-20 min. at room temperature.
5. Add 100 μ l of the TurboFect/siRNA complexes drop-wise to each well.
6. Gently rock the plate to achieve an even distribution of complexes.
7. Incubate at 37°C in a CO₂ incubator.
8. Assay gene silencing 24-72 h later.

siRNA-DNA Cotransfection

For siRNA and plasmid DNA cotransfection follow the general siRNA transfection protocol. For initial experiments, use the recommended amounts of siRNA and plasmid DNA (*see* Table 1). Mix plasmid DNA with the diluted siRNA before adding TurboFect siRNA Transfection Reagent.

(continued on reverse page)

Table 1. Scale-up ratios for transfection of adherent and suspension cells with TurboFect™ siRNA Transfection Reagent.

Tissue culture vessel	96-well plate	48-well plate	24-well plate	12-well plate	6-well plate	60 mm plate
Growth area, cm ² /well	0.3	0.7	2.0	4.0	9.5	20
Volume of complete growth medium for cell plating, ml	0.1	0.25	0.5	1	2	3
Adherent (suspension) cells to seed the day before transfection	0.5-1.2 x 10 ⁴ (2.0 x 10 ⁴)	1.0-3.0 x 10 ⁴ (5.0 x 10 ⁴)	2.0-6.0 x 10 ⁴ (1.0 x 10 ⁵)	4.0-1.2 x 10 ⁵ (2.0 x 10 ⁵)	0.8-2.4 x 10 ⁵ (4.0 x 10 ⁵)	2.0-6.3 x 10 ⁵ (1.0 x 10 ⁶)
Dilution volume for siRNA, µl	25	50	100	200	400	600
Starting quantity of siRNA, pmol (5 nM final siRNA concentration)	0.65	1.5	3	6	12	24
Quantity of siRNA for optimization experiments, pmol (1-25 nM final siRNA concentration)	0.125-3	0.3-7.5	0.6-15	1.25-30	2.5-60	5-120
Volume of TurboFect siRNA Transfection Reagent, µl	0.3	0.5	1	2	4	6
TurboFect volume range for optimization experiments, µl	0.2-0.6	0.3-1	0.8-1.5	1.6-3.5	2-5	5-8
Quantity of DNA for siRNA-DNA cotransfection, µg	0.1	0.25	0.5	1	2	3

Note

The number of cells and volume of transfection reagent required for siRNA transfection were determined using the NIH-3T3 GFP expressing cell line. Quantities may vary depending on the cell type.

Cells successfully transfected using TurboFect siRNA Transfection Reagent include:

Permanently growing cell lines	Primary cell cultures
CHO chinese hamster ovary cells	HLF human lung fibroblasts
HeLa human cervix adenocarcinoma cells	
HR5-CL11 human cervix adenocarcinoma cells, HeLa derivate	
NIH3T3 mouse embryo fibroblasts	

For cell line updates, see www.thermoscientific.com/fermentas

Note

This product or the use of this product is covered by US patent application US20100041739A1 and corresponding counterparts. The purchase of this product includes a non-transferable license to use this product for the purchaser's internal research. All other commercial uses of this product, including without limitation product use for diagnostic purposes, resale of product in the original or any modified form or product use in providing commercial services require a separate license. For further information on obtaining licenses please contact info.fermentas.it@thermofisher.com

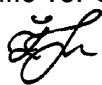
Troubleshooting

Problem	Possible Cause and Solution
Low transfection efficiency	<p>Suboptimal ratio of TurboFect / siRNA. Optimize the amount of transfection reagent added to a fixed amount of siRNA.</p> <p>Suboptimal quantity of siRNA. Optimize the amount of siRNA used for transfection. Keep the amount of TurboFect constant.</p> <p>Poor siRNA quality. Use high quality, sterile siRNA.</p> <p>Tubes contaminated with RNase. Use RNase / DNase-free tubes and pipet tips to prepare TurboFect / siRNA complexes.</p> <p>Suboptimal cell density. Optimize cell plating conditions. Ensure that adherent cells are 70-90 % confluent at the time of transfection. Ensure that suspension cells are in logarithmic growth phase at the time of transfection.</p> <p>Mycoplasma contamination. Mycoplasma infection often results in poor and/or non-reproducible transfection results. Regularly check your cells for mycoplasma infection.</p>
High cellular toxicity	<p>Excessive amount of TurboFect / siRNA complex. Reduce the amount of TurboFect / siRNA complexes used for transfection.</p> <p>Suboptimal incubation conditions. Reduce incubation time of TurboFect / siRNA complexes with the cells. Replace the transfection mixture 3-4 hours later with fresh growth medium.</p> <p>Suboptimal cell density. Increase the plating density of cells used for transfection.</p> <p>Gene silencing affects cell viability. Silencing of the target gene may lead to cellular toxicity. High concentration of siRNA may lead to nonspecific gene silencing and increased toxicity.</p>
Inefficient gene silencing	<p>Poor siRNA quality. Use high quality, sterile siRNA.</p> <p>Suboptimal siRNA design. Use functionally tested siRNA or design siRNA using appropriate design software.</p> <p>Suboptimal assay time. Perform a gene silencing time course following transfection to identify the optimal analysis time of silencing effect.</p> <p>Suboptimal siRNA concentration. Increase the siRNA concentration used for transfection experiments.</p>

CERTIFICATE OF ANALYSIS

Transfection efficiency was tested on HeLa cells by performing plasmid DNA/siRNA cotransfection. 0.5 µg of GFP encoding plasmid DNA, 5 pmol of GFP-specific or nonspecific (control) siRNA and 1 µl of TurboFect siRNA Transfection Reagent were used per 5 x 10⁴ cells seeded a day before transfection in a 24-well plate. The GFP suppression level was estimated by flow cytometry based on the mean fluorescence intensity of cells transfected with nonspecific vs. GFP-specific siRNA. The GFP suppression level was 70±5%.

Quality authorized by:



Jurgita Zilinskiene

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans.

Please refer to www.thermoscientific.com/fermentas for Material Safety Data Sheet of the product.