CERTIFICATE OF ANALYSIS

TurboFect™ Protein Transfection Reagent

125 µl (for 125 transfections in a 24-well plate)
#R1411
Lot: Expiry Date:

Supplied with:
125 µl Enhancer Solution
10 µg (0.5 mg/ml) Fluorescein-labeled Rabbit IgG Control

Storage
Store TurboFect™ Protein Transfection Reagent and Enhancer Solution at 4°C.
Store Fluorescein-labeled Rabbit IgG Control at -20°C in the dark.
Ship at -20°C.

ISO 9001 ISO 14001
www.fermentas.com

Description

TurboFect™ Protein Transfection Reagent* is a sterile solution of a proprietary cationic polymer in water. In combination with the included Enhancer Solution*, the reagent efficiently delivers functionally active proteins, antibodies and peptides into the cytoplasm of cells. Proteins of different sizes and net charge (different pI) can be delivered into various cell types, including primary, adherent and suspension cells. The reagent can be used in the presence or absence of serum.

Fluorescein-labeled Rabbit IgG Control is supplied as a control protein to determine transfection efficiency.

*patent pending

Reagents to be Supplied by the User

0.15 M NaCl, serum-free DMEM, RPMI, OPTI-MEM or other growth medium.

General Considerations

Cell Density

The recommended confluency for adherent cells at the day of transfection is 70-90%.
Suspension cells should be plated at an optimal density ensuring logarithmic growth at the time of transfection.

Incubation Time

The shortest recommended incubation time for the transfection reagent/protein complexes with cells is 2 hours.

Transfection Reagent/Protein Ratio

The volume of transfection reagent used depends on the amount of protein and type of cell 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

Transfection in serum-free medium is recommended for best results. Transfection can be performed in the presence of serum; however, transfection efficiency may be reduced by up to 50%.

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

Quantities and volumes should be scaled-up according to the number of cells/wells to be transfected (*see* Table 1 on reverse page). Subsequent optimization of the quantities of protein and transfection reagent may further increase the transfection efficiency. If toxicity is observed, optimize the transfection reaction by varying the quantities of Enhancer Solution, protein and TurboFect™ transfection reagent.

Note

- Reagents must be added in the order indicated.
- The TurboFect™/protein complexes should be prepared immediately prior to transfection.

1. In a 24-well plate, seed ~$5x10^4$ adherent cells or ~$1x10^5$ suspension cells per well 24 h prior to transfection.

Note

- The recommended confluency for adherent cells on the day of transfection is 70-90%.
- Suspension cells should be plated at an optimal density ensuring logarithmic growth at the time of transfection.

2. Dilute 1 µg of protein in 100 µl of 0.15 M NaCl or serum-free medium.
3. Add 1 µl of Enhancer Solution and mix the solution by vortexing or pipetting.
4. Briefly vortex TurboFect™ Protein Transfection Reagent and add 1 µl of it to the DNA/Enhancer solution. Mix immediately by pipetting or vortexing.
5. Incubate 15-20 min at room temperature.
6. Adherent cells: aspirate the growth medium. Suspension cells: centrifuge the cells at 200 x g for 5 min and aspirate the growth medium.
7. *Optional*. Wash the cells once with serum-free medium.
8. Add 500 µl of serum-free medium to the cells.

Note

- Transfection can be performed in growth medium containing serum. In this case the complete removal of the medium is not required. Aspirate half of the volume used for cell plating.
- Please note that the presence of serum will result in up to a 50% decrease in transfection efficiency.

9. Add 100 µl of the TurboFect™/protein complexes drop-wise to each well.
10. Gently rock the plate to achieve an even distribution of complexes.
11. Incubate for 2 h at 37°C in a CO_2 incubator. Cells can be immediately used for subsequent experiments.
12. If cells are not used immediately, add 500 µl of complete growth medium with 2X serum and incubate until analysis. If toxicity is observed during prolonged incubation, remove the medium containing the TurboFect™/protein complexes 2 hours after transfection, and add 1 ml of complete growth medium.

Note

Prior to analysis, wash the cells thoroughly with serum-free growth medium (PBS or NaCl can also be used). This step removes undelivered protein.

(continued on reverse page)

Table 1. Scale-up ratios for transfection of adherent and suspension cells with TurboFect™ Protein 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	0.2	0.5	1	2	4	6
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)
Volume of serum-free medium, ml	0.1	0.25	0.5	1	2	3
Volume of protein dilution buffer, µl	20	50	100	200	400	600
Amount of protein*, µg	0.25	0.5	1	2	4	6
Volume of Enhancer Solution, µl	0.2	0.5	1	2	4	6
Enhancer Solution volume range for optimization experiments, µl	0.1-0.8	0.25-2.0	0.5-4.0	1-8	2-16	3-24
Volume of TurboFect™ Protein Transfection Reagent, µl	0.2	0.5	1	2	4	6
TurboFect™ volume range for optimization experiments, µl	0.15-0.3	0.25-0.7	0.5-1.4	1-3	2-6	4-8

*****For peptide transfection, double the indicated values.

Note

- The number of cells and volume of reagents required for protein transfection were determined using HeLa and Jurkat cells. Amounts may vary depending on the cell type.
- TurboFect™ Protein Transfection Reagent and Enhancer Solution can be diluted up to 10 times in sterile water immediately before the experiment for accurate pipetting.

Cells successfully transfected using TurboFect™ Protein Transfection Reagent include:

Permanently growing cell lines		Primary cell cultures	
HeLa	human cervix adenocarcinoma cells	BMDC	mouse bone marrow derived dendritic cells
CHO	chinese hamster ovary cells	HLF	human lung fibroblasts
Jurkat	human leukaemic T cells		
HeLa S3	human cervix carcinoma cells		
NIH3T3	mouse embryo fibroblasts		

For cell line updates, *see* www.fermentas.com

Troubleshooting

Problem	Possible Cause and Solution
Low transfection efficiency	Suboptimal reagent/protein ratio. Optimize the amount of transfection reagent used. Suboptimal protein quantity. Optimize the amount of protein used for transfection. Keep the amount of transfection reagent constant. 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. Mycoplasma contamination. Mycoplasma infection often results in poor and/or non-reproducible transfection. Regularly check your cells for mycoplasma infection.
High cellular toxicity	Suboptimal protein quantity. Titrate the amount of protein used for transfection. The amount of protein delivered can be reduced by decreasing amount of Enhancer Solution. Toxic protein. Lower the quantity of protein delivered into cells by reducing the amount of protein used for transfection or by reducing amount of Enhancer Solution used. In addition, cells can be transfected with the included control protein. Suboptimal incubation conditions. Replace the transfection mixture after a 2 hour incubation with complete growth medium. Reduce the incubation time of reagent/protein complexes with the cells. Suboptimal cell density. Increase the plating density of cells used for transfection.

QUALITY CONTROL

Transfection efficiency was tested on HeLa cells using 1 µg of Fluorescein-labeled Rabbit IgG, 1 µl of Enhancer Solution and 1 µl of TurboFect™ Protein Transfection Reagent per 5 x 10^4 cells seeded a day before transfection in 24-well plate. Transfection efficiency, i.e. the percentage of transfected cells, is 80±10% as estimated by flow cytometry.

Quality authorized by:  Jurgita Zilinskiene