TurboFect™ *in vitro* Transfection Reagent

#R0531
1 ml (for 300-500 *in vitro* transfections)

Description

TurboFect™ *in vitro* Transfection Reagent* is a sterile solution of a cationic polymer in water. The polymer forms compact, stable, positively charged complexes with DNA. These complexes protect DNA from degradation and facilitate gene delivery into eukaryotic cells. TurboFect™ is ideal for transfection of a variety of cells, including primary and difficult-to-transfect cells. Transfection can be performed in the presence or absence of serum. TurboFect™ demonstrates superior transfection efficiency and minimal toxicity when compared to lipid-based or other polymer-based transfection reagents.

Storage

Store at 4°C.

Reagents to be Supplied by the User

Serum-free DMEM, RPMI or other growth medium.
The presence of antibiotics in the medium has no effect on transfection efficiency.

* patent pending.

General Considerations

DNA Quality Requirements

DNA quality is critical for successful transfection. An A_{260}/A_{280} ratio of 1.8 or higher is recommended. Endotoxin-contaminated DNA may result in inefficient transfection and cause unacceptably high cellular toxicity.

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 their logarithmic growth at the time of transfection.

Incubation Time

Transient transgene expression takes place within 2-72 hours after transfection. The optimal incubation time depends on the cell type, promoter strength and expression product, and must be determined experimentally.

Choice of Promoter

High transfection efficiency depends both on the transgene promoter and on the cell line used. Cytomegalovirus (CMV) promoter is commonly used for high gene expression in a variety of cell lines. Other promoters, such as those from simian virus (SV40) and from Rous sarcoma virus (RSV) can also be used.

Transfection Reagent/DNA Ratio

The volume of transfection reagent used depends on the amount of DNA, transgene and cells to be transfected. The ratios presented in the protocols below are starting amounts and can be further optimized for best results.

Transfection in the Presence of Serum

The transfection efficiency with TurboFect™ *in vitro* transfection reagent is equally high in the presence of serum. This is not the case with many other transfection reagents.

Antibiotics

Antibiotics do not interfere with both DNA/TurboFect™ complex formation and cell transfection.

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

The protocol is optimized for transfection in 24-well plate format. Quantities and volumes should be scaled-up according to the number of cells/wells to be transfected (see Table 1).

❶ In each well, seed ~5x10^4 adherent cells or ~5x10^5 suspension cells in 1 ml of growth medium 24 hours prior to transfection.

Notes

– The recommended confluency for adherent cells on the day of transfection is 70-90%.

– Suspension cells should be in logarithmic growth phase at the time of transfection.

❷ Dilute 1 µg of DNA in 100 µl of serum-free DMEM or other serum-free growth medium.

❸ Add 2 µl of TurboFect™ to the diluted DNA and mix by pipetting.

❹ Incubate 15-20 minutes at room temperature.

Notes

– Prepare immediately prior to transfection.

– We recommend starting with 1 µg of DNA and 2 µl of TurboFect™ per well in a 24-well plate (see scale-up Table on next page).

– Subsequent optimization may further increase transfection efficiency depending on the cell line and transgene used.

❺ Add 100 µl of the TurboFect™/DNA mixture drop-wise to each well. Do not remove the growth medium from the cells.

❻ Gently rock the plate to achieve even distribution of the complexes.

❼ Incubate at 37°C in a CO_2 incubator.

❽ Analyze transgene expression 24-48 hours later. For stable transfection, cells should be grown in selective medium for 10-15 days.

Protocol for Reverse Transfection of Adherent and Suspension Cells In a 24-well Plate

The protocol is optimized for transfection in 24-well plate format. Quantities and volumes should be scaled-up according to the number of cells/wells to be transfected (see Table 1).

❶ Dilute 1 µg of DNA in 100 µl of serum-free DMEM or other serum-free growth medium.

❷ Add 2 µl of TurboFect™ to the diluted DNA and mix by pipetting.

❸ Incubate 15-20 minutes at room temperature.

❹ Evenly distribute 100 µl of the TurboFect™/ DNA mixture to the bottom of each well of a 24-well plate.

Notes

- Prepare immediately prior to transfection
- We recommend starting with 1 µg of DNA and 2 µl of TurboFect™ per well in a 24-well plate (see scale-up Table on next page).
- Subsequent optimization may further increase transfection efficiency depending on the cell line and transgene used.

❺ Gently layer 1 ml of ~10^5 adherent cells or ~10^6 suspension cells per well on top of the TurboFect™/ DNA mixture.

❻ Incubate at 37°C in a CO_2 incubator.

❼ Analyze transgene expression 24-48 hours later.

Note

Plates can be centrifuged for 2-5 min at 200xg to facilitate sedimentation of cells to the bottom of the plate.

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

Tissue culture vessel	Growth area, cm^2/well	Volume of medium, ml	Adherent (suspension) cells to seed the day before transfection*	Amount of DNA		Volume of TurboFect™, µl*	
				µg**	µl***	Recom-mended	Range
96-well plate	0.3	0.2	0.5-1.2 x 10^4 (2.0 x 10^4)	0.2	20	0.4	0.3-0.6
48-well plate	0.7	0.5	1.0-3.0 x 10^4 (5.0 x 10^4)	0.5	50	1.0	0.5-1.4
24-well plate	2.0	1.0	2.0-6.0 x 10^4 (1.0 x 10^5)	1.0	100	2.0	1.0-2.8
12-well plate	4.0	2.0	0.4-1.2 x 10^5 (2.0 x 10^5)	2.0	200	4.0	2.6-6.0
6-well plate	9.5	4.0	0.8-2.4 x 10^5 (4.0 x 10^5)	4.0	400	6.0	4.0-8.0
60 mm plate	20	6.0	2.0-6.3 x 10^5 (1.0 x 10^6)	6.0	600	12.0	8.0-16.0

Note

* These numbers were determined using HeLa and Jurkat cells. Actual values depend on the cell type.

** Amount of DNA and TurboFect™ *in vitro* Transfection Reagent used may require optimization.

*** The volume of DNA should be 1/10 of the volume of the culture medium used for dilution of the DNA.

Quality Control

Transfection efficiency was tested on HeLa cells using 1 µg of eGFP expressing plasmid and 2 µl of TurboFect™ per 5 x 10^4 cells in 24-well plate. Transfection efficiency, i.e. the percentage of transfected cells, is 91.0±9% as estimated by flow cytometry.

Quality authorized by:  Jurgita Zilinskiene

Troubleshooting

Problem	Possible Cause and Solution
Low transfection efficiency	**Suboptimal reagent/DNA ratio.** Optimize the amount of transfection reagent added to the fixed amount of DNA. **Suboptimal quantity of DNA.** Optimize the amount of DNA used for transfection. Keep the transfection reagent/DNA ratio constant. **Poor DNA quality.** Use high quality DNA with an A_{260}/A_{280} ratio greater than 1.8. **Suboptimal cell confluency.** Optimize cell plating conditions. Ensure that adhered 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 in cell culture often results in poor and/or non-reproducible transfection. Regularly check your cells for mycoplasma infection.
High cellular toxicity	**Toxic transgene.** Verify if the expressed transgene is toxic. **Suboptimal incubation conditions.** Reduce incubation time of the polyplexes with the cells. Replace the transfection mixture 3-6 hours later with fresh growth medium. **Suboptimal quantity of DNA.** Reduce the quantity of DNA used for transfection. **Cell density is too low.** Increase the plating density of cells used for transfection.

Cells successfully transfected with TurboFect™ include:

Permanently growing cell lines

- **Cos-7** african green monkey kidney cells
- **HeLa** human cervix adenocarcinoma cells
- **CHO** chinese hamster ovary cells
- **HEK293** human embryonic kidney cells
- **B50** rat nervous tissue neuronal cells
- **Calu1** human lung epidermoid carcinoma cells
- **RAW264** mouse leukaemic monocyte-macrophage cells
- **WEHI** mouse B cell lymphoma cells
- **MDCK** Madin Darby Canine Kidney cells
- **Raji** human Burkitt's lymphoma cells
- **COLO** human colon adenocarcinoma cells
- **Jurkat** human leukaemic T cells
- **Sp2/Ag14** mouse myeloma cells
- **HeLa S3** human cervix carcinoma cells
- **Hep2C** human larynx carcinoma cells
- **L929** mouse connective tissue fibroblasts
- **NIH3T3** mouse embryo fibroblasts

Primary cell cultures

- Rat fibroblasts
- Mouse bone marrow derived dendritic cells
- Mouse bone marrow derived macrophages
- HLF human lung fibroblasts

For cell line updates, see www.fermentas.com/catalog/transfection.

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 or animals.

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