INSTRUCTIONS

TurboFect™ Transfection Reagent

R0532 R0533 R0534

2444.0

Number	Description
R0533	**TurboFect Transfection Reagent,** 200µL, for 60-100 *in vitro* transfections
R0534	**TurboFect Transfection Reagent,** 5 × 200µL, for 300-500 *in vitro* transfections
R0532	**TurboFect Transfection Reagent,** 5 × 1mL, for 1500-2500 *in vitro* transfections

Storage: Upon receipt store at 4°C. Product shipped at ambient temperature.

Introduction

The Thermo Scientific TurboFect Transfection Reagent is a sterile solution of cationic polymers in water. The polymers and deoxyribonucleic acid (DNA) form compact, stable, positively charged complexes, which protect DNA from degradation and facilitate gene delivery into eukaryotic cells. The transfection reagent is ideal for transfection of primary cells, difficult-to-transfect cells and other types of cells with transfection being possible in the presence or absence of serum. The transfection reagent demonstrates superior transfection efficiency and minimal toxicity when compared to lipid-based or other polymer-based transfection reagents.

Important Product Information

- DNA quality is critical for successful transfection. For excitation/emission absorbance at 260/280nm, a ratio of 1.8 or higher is recommended.
- Endotoxin-contaminated DNA results in inefficient transfection and can cause high cellular toxicity.
- At the time of transfection, the optimal confluency for adherent cells is 70-90%.
- Plate suspension cells at an optimal density to ensure logarithmic growth at the time of transfection.
- Transient transgene expression occurs within 2-72 hours after transfection.
- The optimal incubation time must be empirically determined and depends on the cell type, promoter strength and expression product.
- High transfection efficiency depends on the transgene promoter and the cell line used.
- Cytomegalovirus (CMV) promoter is commonly used for high gene expression in a variety of cell lines; however, other promoters such as simian virus (SV40) and Rous sarcoma virus (RSV) can also be used.
- 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 may be optimized.
- Antibiotics do not interfere with transfection reagent/DNA complex formation, cell transfection or transfection efficiency.

Protocol for Transfection of Cells

A. Material Required

- **Growth medium:** Serum-free DMEM, RPMI or other growth medium

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

Note: The protocol is optimized for transfection in a 24-well plate format. At the time of transfection, the optimal confluency for adherent cells is 70-90%. Suspension cells should be in logarithmic growth phase at the time of transfection. For best results, start with 1µg of DNA and 2µL of transfection reagent per well in a 24-well plate (See Table 1). Subsequent optimization can further increase transfection efficiency, depending on the cell line and transgene used.

1. In each well, seed ~5×10^4 adherent cells or ~5×10^5 suspension cells in 1mL of growth medium 24 hours before transfection.

   Note: Prepare transfection reagent immediately before transfection.
2. Dilute 1µg of DNA in 100µL of serum-free DMEM or other serum-free growth medium.
3. Briefly vortex the transfection reagent and add 2µL to the diluted DNA. Mix immediately by pipetting or vortexing.
4. Incubate 15-20 minutes at room temperature.
5. Add 100µL of the transfection reagent/DNA mixture drop-wise to each well. Do not remove the growth medium from the cells before adding the transfection reagent/DNA mixture.
6. Gently rock the plate to achieve even distribution of the complexes immediately after adding the transfection reagent.
7. Incubate at 37°C in a CO_2 incubator.
8. Analyze transgene expression after 24-48 hours. For stable transfection, grow cells in selective medium for 10-15 days.

C. Protocol for Reverse Transfection of Adherent and Suspension Cells in a 24-well Plate

Note: The protocol is optimized for transfection in a 24-well plate format. Scale-up quantities and volumes according to the number of cells/wells to be transfected (See Table 1). For best results, start with 1µg of DNA and 2µL of transfection reagent per well in a 24-well plate. Subsequent optimization can further increase transfection efficiency, depending on the cell line and transgene used.

Note: Prepare transfection reagent immediately before transfection.

1. Dilute 1µg of DNA in 100µL of serum-free DMEM or other serum-free growth medium.
2. Briefly vortex the transfection reagent and add 2µL to the diluted DNA. Mix immediately by pipetting or vortexing.
3. Incubate 15-20 minutes at room temperature.
4. Evenly distribute 100µL of the transfection reagent/ DNA mixture at the bottom of the well of a 24-well plate.
5. Gently layer 1mL of ~10^5 adherent cells or ~10^6 suspension cells per well on top of the transfection reagent/DNA mixture.
6. Incubate at 37°C in a CO_2 incubator.
7. Analyze transgene expression after 24-48 hours.

   Note: Plates can be centrifuged for 2-5 minutes at 200 × *g* to collect cells at the bottom of the plate.

Table 1. Scale-up ratios for transfection of adherent and suspension cells using Thermo Scientific TurboFect Transfection Reagent.

Tissue culture plate	Growth area (cm^2/well)	Volume of medium (mL)	No. of adherent (suspension) cells to seed the day before transfection*	Amount of DNA (µg)	Amount of DNA (µL)**	Volume of TurboFect Transfection Reagent (µL) Recommended	Volume of TurboFect Transfection Reagent (µL) Range
96-well plate	0.3	0.2	0.5-1.2 × 10^4 (2.0 × 10^4)	0.2	20	0.4	0.3-0.6
48-well plate	0.7	0.5	1.0-3.0 × 10^4 (5.0 × 10^4)	0.5	50	1.0	0.5-1.4
24-well plate	2.0	1.0	2.0-6.0 × 10^4 (1.0 × 10^5)	1.0	100	2.0	1.0-2.8
12-well plate	4.0	2.0	0.4-1.2 × 10^5 (2.0 × 10^5)	2.0	200	4.0	2.6-6.0
6-well plate	9.5	4.0	0.8-2.4 × 10^5 (4.0 × 10^5)	4.0	400	6.0	4.0-8.0
60mm plate	20	6.0	2.0-6.3 × 10^5 (1.0 × 10^6)	6.0	600	12.0	8.0-16.0

*Values for suspension cells are in parentheses.
**The volume of DNA should be 1/10 the volume of the culture medium used for dilution of the DNA.
Note: These numbers were determined using HeLa and Jurkat cells. Actual values depend on the cell type. The amount of DNA and TurboFect Transfection Reagent used may require optimization.

Troubleshooting

Problem	Possible Cause	Solution
Low transfection efficiency	Suboptimal transfection 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 amount of transfection reagent constant
	Poor DNA quality	Use high-quality DNA with an absorbance ratio greater than 1.8 at 260/280nm
	Suboptimal cell confluency	Optimize cell plating conditions
		Ensure 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	Regularly check cells for mycoplasma infection

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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 3-4 hours later with new growth medium
	Excess amount of DNA	Reduce the quantity of DNA used for transfection
	Cell density was too low	Increase the plating density of cells used for transfection
	Endotoxin or other toxic materials were present with transgene	Ensure transgene is free of toxic substances
		Repeat insertion of gene into new toxin-free plasmid preparation

Additional Information

A. Cells successfully transfected with Thermo Scientific TurboFect Transfection Reagent.

Permanently growing cell lines		**Primary cell cultures**
Cos-7	African green monkey kidney cells	Rat fibroblasts
HeLa	Human cervix adenocarcinoma cells	Mouse bone marrow-derived dendritic cells
CHO	Chinese hamster ovary cells	Mouse bone marrow-derived macrophages
HEK293	Human embryonic kidney cells	Human lung fibroblasts (HLF)
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	

Related Thermo Scientific Products

16146-89 **Pierce® Luciferase Assay Kits and Reagents**

88273 **High Capacity Endotoxin Removal Spin Columns, 0.1mL,** 5/pkg

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