TransFectin™ Lipid Reagent

0.5 ml	170-3350
1.0 ml	170-3351
5 x 1.0 ml	170-3352

For Research Use Only
Store at 4°C

Storage and Stability

TransFectin Lipid Reagent is shipped on ice. Store at 4° C upon receipt. **Do not store below 0°C**. TransFectin is stable for 6 months from date of purchase when stored at 4° C.

Contents

TransFectin contains either 0.5 ml (Catalog Number 170-3350), 1.0 ml (Catalog Number 170-3551), or 5 x 1.0 ml (Catalog Number 170-3352) of a proprietary lipid formulation. One milliliter is generally sufficient for 125 to 200 cell transfections in 35 mm plates. TransFectin comes as a 1.5 mg/ml solution.

Reagent

TransFectin Lipid Reagent is a mixture of a proprietary cationic compound and a co-lipid (1,2-Dioleoyl-*sn*-Glycero-3-Phosphoethanolamine). These compounds have been optimized for intracellular delivery of nucleic acids into cultured mammalian cells **in the presence of serum at cell densities from 50% to greater than 90%**. For most cell lines, high levels of expression can be obtained using concentrations of TransFectin and DNA suggested in this manual for given sizes of plates/wells. If presently using a cationic lipid for transfection, start using TransFectin at current conditions and also try reducing and increasing the volume of TransFectin from 25 to 50% of current amount. For best results it is important to determine the optimal amount of DNA and lipid for any given cell line.

Recommendations for Best Results

- For most cell lines use a ratio of DNA (µg) to lipid (µl) of 1:2–1:3 as a starting point to optimize conditions.
- Invert the tube to mix contents before using.
- TransFectin is designed to work in media containing serum but can be used in the absence of serum.
- Use sterile polystyrene plastic ware (e.g. 12 x 75 mm tubes or multi-well trays) to prepare the plasmid solutions and lipid solutions. Polystyrene is recommended because cationic lipid-plasmid complexes may bind to polypropylene.

Optimization

Determining the optimum conditions for transfection efficiency is essential for maximizing gene expression and minimizing cellular toxicity. The two most important parameters to optimize for any given culture vessel and cell density are the concentration of TransFectin and the amount of nucleic acid. **See Table 1 for suggested reagent and DNA concentrations for different culture vessel sizes**. In general, gene expression will increase, plateau and then decrease with increasing concentrations of TransFectin. This decrease in gene expression correlates with reduced cell viability. As increasing amounts of plasmid are added to the cells, gene expression will increase, then plateau or even decrease since the amount of DNA used can directly affect toxicity. If toxicity is encountered, try reducing lipid or DNA amounts used in transfection. The concentration of TransFectin and the amount of plasmid required for maximal expression may vary from one cell line to another.

Table 1. Suggested Reagent Quantities for Different Sizes of Plates/Wells

Culture Vessel Size	Surface Area (cm^2)	Volume of Plating Media	Plasmid DNA	Volume of Serum Free Medium	TransFectin Reagent
96 well	0.32	0.1 ml	50–200 ng	25 µl	0.1–0.6 µl
24 well	1.9	0.5 ml	0.25–1.0 µg	50 µl	0.25–4.0 µl
12 well	3.8	1.0 ml	0.50–2.0 µg	100 µl	2.0–8.0 µl
6 well/35mm	9.2	2.0 ml	1.0– 4.0 µg	250 µl	5.0–15 µl
60 mm	21	5.0 ml	2.0–8.0 µg	500 µl	15–20 µl
100 mm	60	10.0 ml	12–36 µg	1.5 ml	40–60 µl

Protocol for Transfection of Adherent Cells
(24 Well Plates)

1. The day before transfection, inoculate 24-well plates with an appropriate number of cells in serum-containing medium such that they will be 50 to 90% confluent the following day. For most cell lines, plating 0.5 to 8.0 x 10^5 cells in 0.5 ml of medium should be appropriate. Incubate the cells at 37°C in a 5% CO_2 incubator overnight.
2. For each well, prepare plasmid in 50 µl of serum-free medium. Use 0.25 to 1.0 µg of plasmid DNA for each 50 µl of serum-free medium.
3. Prepare the TransFectin reagent in 50 µl of serum-free medium. Use 0.25 to 4.0 µl for each well as a starting point for optimizing the reaction.
4. Mix the DNA and TransFectin solutions together. Gently mix by tapping or pipetting. Incubate 20 minutes at room temperature.
5. Add 100 µl of the DNA–TransFectin complexes directly to cells in serum-containing medium. Swirl gently. Incubate the cells at 37°C in a 5% CO_2 incubator. Additional medium may be added 4 to 6 hours after addition of complexes.
6. For transient expression, assay for reporter gene activity 24 to 48 hours after the transfection.
7. For stable expression of the transfected plasmid sequence, remove transfection medium 24 hours after transfection and trypsinize the cells. Transfer the cells to a fresh plate with growth medium containing no selective agent. The following day, replace with new medium containing the selective agent. Continue incubating for 1 to 2 weeks to allow growth of the cells expressing the transgene.

Protocol for Transfection of Suspension Cells
(24 Well Plates)

1. The day before transfection, dilute the cells such that they will be in log phase growth the following day. For most cell lines, inoculating 0.75 to 8.0 x 10^5 cells per 0.5 ml of medium should be appropriate. Incubate the cells overnight at 37°C in a 5% CO_2 incubator.
2. For each well, prepare plasmid in 50 µl of serum-free medium. Use 0.25 to 1.0 µg of plasmid for each 50 µl of medium.
3. Prepare TransFectin in 50 µl of serum-free medium for each well. Use 0.25 to 4.0 µl of lipid for each 50 µl of medium.
4. Mix the plasmid and TransFectin solutions together. Gently mix by tapping or pipetting. Incubate 20 minutes at room temperature.
5. Add the 100 µl of DNA–TransFectin complexes to the cell suspension; rock the plate gently to ensure adequate mixing of the solutions.
6. Incubate the cells at 37°C in a 5% CO_2 incubator. Additional medium may be added 4 to 6 hours after addition of complexes.
7. For transient expression, assay reporter gene activity 24–48 hours after transfection.

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