TECHNICAL MANUAL

ViaFect™ Transfection Reagent

Instructions for Use of Products
E4981, E4982 and E4983

Revised 7/17
TM409

ViaFect™ Transfection Reagent

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1. Description

ViaFect™ Transfection Reagent[a] is a novel formulation designed to transfect DNA into a wide variety of cell lines with high efficiency and low toxicity. ViaFect™ Transfection Reagent has been shown to transfect cell lines that are traditionally thought of as difficult to transfect (such as hematopoietic, primary and iPSC stem cell lines) with relatively high efficiency. The protocol does not require removal of serum or culture medium and does not require washing or changing of medium after introducing the reagent/DNA complex. Additionally, ViaFect™ Transfection Reagent does not contain any animal-derived components.

1. Description (continued)

The cell lines listed in Table 1 have been transfected successfully by Promega Corporation.

Table 1. Cell Lines Successfully Transfected Using the ViaFect™ Transfection Reagent.

HEK-293	HeLa	MCF7	U-2 OS
A549	LNCaP	PC-3	HCT 116
HT-29	Hep G2	Huh-7	K-562
Jurkat	C2C12	RAW 264.7	NIH/3T3
COS-7	CHO	H9c2	PC-12
TF-1	HUVEC	Cardiomyocytes	THP-1
MDA-MB-453	Raji	HepaRG®	Ba/F3
U-937	L-929	5637	

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
ViaFect™ Transfection Reagent	**0.75ml**	**E4981**
	2 × 0.75ml	**E4982**
	0.2ml	**E4983**

Storage Conditions: Store ViaFect™ Transfection Reagent at +2°C to +10°C. Do not freeze. Handle in a biological safety cabinet to minimize contamination.

Formulation and Packaging: ViaFect™ Transfection Reagent is a cationic delivery reagent in aqueous solution filtered through a 0.2µm filter. The reagent does not contain ingredients of human or animal origin.

Special Handling: Allow ViaFect™ Transfection Reagent to reach room temperature, and mix briefly by inverting prior to use. Do not dispense ViaFect™ Transfection Reagent into aliquots. It is best to dilute ViaFect™ Transfection Reagent directly into medium without contacting the side of the tube.

3. General Considerations

Successful transfection involves optimizing the ViaFect™ Transfection Reagent:DNA ratio, amount of DNA used, complexing time, type of cells and medium used, etc. For a detailed optimization protocol, see Section 4.E. Plasmids with reporter gene functions can be used to monitor transfection efficiencies. An ideal reporter gene product is unique to the cell, can be expressed from plasmid DNA and can be assayed conveniently. Generally, such assays are performed 24–48 hours after transfection. Promega offers reporter genes and assays for luciferase, green fluorescent protein (hMGFP), chloramphenicol acetyltransferase (CAT) and β-galactosidase as well as reagents for covalent protein labeling (HaloTag® protein).

3.A. Ratio of Transfection Reagent:DNA

For successful transfection of DNA into cultured cells, the ratio of ViaFect™ Transfection Reagent:DNA must be optimized. Ratios ranging from 2:1 to 6:1 of ViaFect™ Transfection Reagent:DNA (µl reagent:µg DNA) work well with many cell lines, but ratios outside of this range may be optimal for other cell types or applications.

3.B. DNA

Plasmid DNA for transfections should be free of protein, RNA and chemical contamination (A_{260}/A_{280} ratio of 1.7–1.9). The PureYield™ Plasmid Purification Systems will provide DNA of sufficient quality for most cell systems. Prepare purified DNA in sterile water or TE buffer at a final concentration of 0.2–1mg/ml. The optimal amount of DNA to use in the transfection will vary widely, depending upon the type of DNA and target cell line used. We recommend initially testing 50–100ng of DNA per well in a 96-well plate format at a ViaFect™ Transfection Reagent:DNA ratio of 3:1 or 4:1 for adherent cell lines or 2:1 for suspension cell lines. Increasing the amount of DNA does not necessarily result in higher transfection efficiencies. To help decrease toxicity if observed, especially in primary cells, lowering the DNA concentration may be helpful.

3.C. Time

The time required to form the ViaFect™ Transfection Reagent:DNA complex is 5–20 minutes at room temperature. The optimal time for complex formation varies for each cell line. Incubate transfected cells for 24–48 hours before assaying to allow time for expression of transfected DNA.

3.D. Serum

Transfection protocols often require serum-free conditions for optimal performance because serum can interfere with many commercially available transfection reagents. ViaFect™ Transfection Reagent can be used in transfection protocols in the presence of serum, allowing transfection of cell types that require continuous exposure to serum, such as primary cell cultures.

3.E. Antibiotics

Although antibiotics can be used during the culture of cell lines, the presence of antibiotics during transfection may adversely affect transfection efficiency and the overall health of transfected cells. We do not recommend using antibiotics in the transfection medium unless previously tested in the cell type being transfected.

3.F. Stable Transfection

ViaFect™ Transfection Reagent can be used to produce stable transfectants. However, we recommend optimizing transfection conditions using transient transfection studies prior to selecting for stable transfectants.

4. Recommended Protocol

Figure 1 provides an overview of the transfection procedure. We recommend using a 96-well plate format to optimize transfection conditions for a particular cell type.

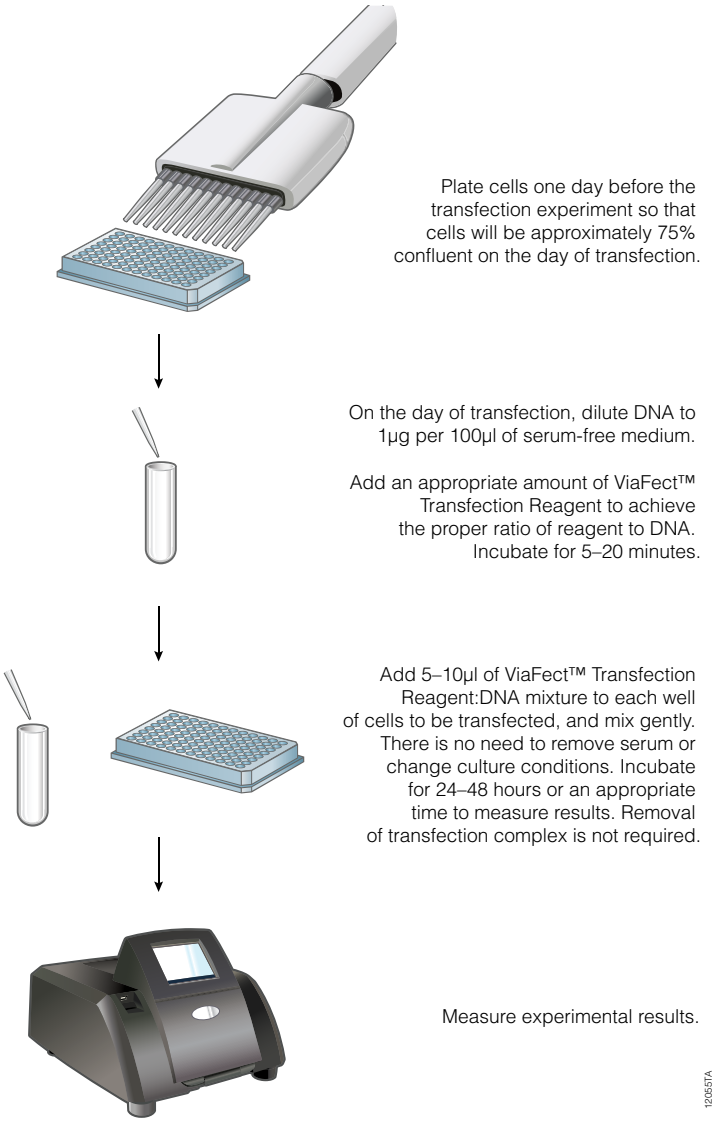

Figure 1. Overview of adherent cell transfection protocol for a 96-well plate.

Materials to Be Supplied by the User

- cell culture medium with serum appropriate for the cell type being transfected
- serum-free cell culture medium for complex formation (such as Opti-MEM® I reduced-serum medium)
- 96-well or other culture plates
- U- or V-bottom dilution plates or microcentrifuge tubes

4.A. Plating Cells

Plate adherent cells one day before transfection so cells are approximately 75% confluent on the day of transfection. Suspension cells can be plated on the day of transfection. As a general guideline, plate 1–2 × 10^4 adherent cells or 2—10 × 10^4 suspension cells in 100µl per well of a 96-well plate. Adjust cell numbers proportionately for different size plates (see Table 2). To prepare cells, collect enough cells to complete the transfection experiment and centrifuge for 5 minutes at 200 × *g* in a swinging-bucket rotor. Suspend the cell pellet to an appropriate concentration in medium, and then plate.

ViaFect™ Transfection Reagent typically demonstrates low toxicity at low cell densities and therefore can be used on cells at low confluence. This allows the use of transfected cells in multiday assays where overly confluent cells could skew results.

Table 2. Area of Culture Plates for Cell Growth.

Plate Size	Growth Area (cm^2)[1]	Relative Area[2]
96-well	0.32	1X
24-well	1.88	5X
12-well	3.83	10X
6-well	9.4	30X
35mm	8.0	25X
60mm	21	65X
100mm	55	170X

[1]This information was calculated for Corning culture dishes.

[2]Relative area is expressed as a factor of the total growth area of the 96-well plate recommended for optimization studies. To determine the proper adherent cell plating density, multiply 1–2 × 10^4 cells by this factor.

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4.B. Preparing the ViaFect™ Transfection Reagent

1. Before use, allow the vial of ViaFect™ Transfection Reagent to reach room temperature.
2. Mix by inverting briefly.

4.C. General Transfection Protocol

We strongly recommend that you optimize transfection conditions for each cell line. If you have optimized transfection parameters as described in Section 4.E, use the empirically determined conditions for your experimental transfections. If you choose not to optimize transfection parameters, use the general conditions recommended below.

1. The total volume of transfection complex (medium, DNA and ViaFect™ Transfection Reagent) to add per well of a 96-well plate is 5–10µl (Table). The following protocol is a guideline for transfecting approximately 10–20 wells, depending on the volume of ViaFect™ Transfection Reagent:DNA mixture used. For additional wells, scale volumes accordingly. To a sterile tube or U- or V-bottom plate, add 90–99µl of serum-free medium prewarmed to room temperature so that the final volume after adding the DNA is 100µl. Add 1µg of plasmid DNA to the medium and mix. For a 3:1 ViaFect™ Transfection Reagent:DNA ratio, add 3µl of ViaFect™ Transfection Reagent, and mix immediately.

Table 3. Total Volume of Medium, DNA and ViaFect™ Transfection Reagent for 96-Well Plates at a 3:1 ViaFect™ Transfection Reagent:DNA Ratio.

Plate Size[1]	Total Transfection Volume (per well)[2]	Amount of DNA (per well)[2,3]	Amount of ViaFect™ Transfection Reagent (per well)[2]
96-well	5–10µl	0.05–0.1µg	0.15–0.3µl

[1]See Table 5 for reagent volumes to use for scaling up to larger wells or flasks.

[2]See Table 4 for ViaFect™ Transfection Reagent volumes at different reagent:DNA ratios.

[3]DNA amount also can be optimized; 1µg/100µl is a recommended starting point.

2. Incubate the ViaFect™ Transfection Reagent:DNA mixture for 5–20 minutes at room temperature.

   Optional: Add mixture to cells without an incubation period.

   Note: Longer incubations may adversely affect transfections.
3. Add 5–10µl of the ViaFect™ Transfection Reagent:DNA mixture per well to a 96-well plate containing 100µl of cells in growth medium. We suggest 10µl of mixture as a starting point. Mix gently by pipetting or using a plate shaker for 10–30 seconds. Return cells to the incubator for 24–48 hours.

   Note: The total growth medium volume may vary depending on well format and your laboratory's common practices.
4. Measure transfection efficiency using an assay appropriate for the reporter gene. For transient transfection, cells are typically assayed 24–48 hours after transfection.

4.D. Protocol for Stable Transfection

The goal of stable transfection is to isolate and propagate individual clones containing transfected DNA. Therefore, it is necessary to distinguish nontransfected cells from those that have taken up the exogenous DNA. This screening can be accomplished by drug selection when an appropriate drug resistance marker is included in the transfected DNA.

Typically, cells are maintained in nonselective medium for 1–2 days post-transfection and then switched to selection medium (medium containing the appropriate drug). The use of selection medium is continued for 2–3 weeks, with frequent changes of medium to eliminate dead cells and debris until distinct colonies can be visualized. Individual colonies are then trypsinized and transferred to flasks for further propagation or to multiwell plates for limited dilution cloning in the presence of selection medium.

Several drug-selection markers are commonly used for long-term transfection studies. For example, cells transfected with recombinant vectors containing the bacterial gene for aminoglycoside (i.e., neomycin) phosphotransferase can be selected for stable transfection in the presence of the drug G-418 (1). Similarly, expression of the gene for hygromycin B phosphotransferase from the transfected vector will confer resistance to the drug hygromycin B (2). Promega offers vectors conferring resistance to G-418, hygromycin B or puromycin.

Before using a particular drug for selection purposes, it is necessary to determine the concentration of drug required to kill the nontransfected cells. As this may vary from one cell type to another, construct a kill curve for nontransfected cells using varying concentrations of the drug to determine the amount needed to select resistant clones. The optimal drug concentration is generally one that induces cell death in >90% of nontransfected cells within 5–7 days.

For stable transfections, cells should be transfected with a plasmid containing a drug resistance marker using the transfection protocols outlined in Sections 4.C and 4.E. **Optional:** Transfect cells with DNA that does not contain the drug-resistance gene as a negative control for drug selection.

1. Forty-eight hours post-transfection, harvest adherent cells and plate at several different dilutions (e.g., 1:2, 1:5, 1:10) in selective medium.
2. For the next 14 days, replace the selective medium every 3–4 days.
3. During the second week, monitor cells for distinct colonies of surviving cells. Complete cell death should occur in cells transfected with the negative control plasmid.
4. Transfer individual clones by standard techniques (e.g., using cloning cylinders or limiting dilution cloning) to 96-well plates, and continue to maintain cultures in selective medium.

   Note: If single clones are not required, pools of stable transfectants can be maintained and frozen.

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4.E. Transfection Optimization

We strongly recommend that you optimize transfection conditions for each cell line. For this initial optimization, we recommend using 10–100ng of DNA per well at various ratios of ViaFect™ Transfection Reagent:DNA (Table 4). We recommend using standard growth conditions with cells in 96-well plates.

Table 4. Optimization Protocol Using Varying Ratios of ViaFect™ Transfection Reagent:DNA.

	Ratio of ViaFect™ Transfection Reagent:DNA				
	1.5:1	**2:1**	**3:1**	**4:1**	**6:1**
Medium to a final volume of[1]	100µl	100µl	100µl	100µl	100µl
DNA amount[2]	1µg	1µg	1µg	1µg	1µg
Volume of ViaFect™ Transfection Reagent	1.5µl	2µl	3µl	4µl	6µl

[1]These volumes are sufficient for 20 wells (5µl/well) or 10 wells (10µl/well) of a 96-well plate for each ratio.

[2]DNA amount also can be optimized; 1µg/100µl is a recommended starting point.

1. For a 96-well plate, the total volume of medium and cells per well prior to transfection should be 100µl. The volume of DNA and ViaFect™ Transfection Reagent complex added should be optimized; 5–10µl per well is a good starting range, but other volumes may be optimal depending on the transfection parameters. Calculate the total amount of complex needed for each transfection condition (Table 4). In a sterile tube or a U- or V-bottom plate, combine the indicated amount of serum-free medium (prewarmed to room temperature) and plasmid DNA; mix well. Add the indicated amount of ViaFect™ Transfection Reagent (also prewarmed to room temperature), and mix by pipetting.
   If desired, ViaFect™ Transfection Reagent also can be diluted in serum-free medium prior to mixing with diluted DNA, but it must be used within 30 minutes.
2. Allow the ViaFect™ Transfection Reagent:DNA complex to incubate at room temperature for 5–20 minutes.
3. Add 5–10µl of complex per well to 96-well plate containing 100µl of cells in growth medium. Mix gently by pipetting or using a plate shaker for 10–30 seconds. Return plate to the incubator. For many reporter systems (luciferase, CAT, β-galactosidase, etc.) a 24- to 48-hour incubation is sufficient.
4. Check the transfection efficiency using an assay appropriate for the reporter system. For multiplexing two reporter assays or one reporter assay with cell viability or toxicity measurement, see Section 4.F.

4.F. Scaling Reagent Volumes

If using larger plate formats or flask sizes than a 96-well plate or T75-flask, respectively, follow the same protocol as previously described but with proportionally scaled-up volumes of reagents. Adjust the total volumes according to the total volume of cells in media, leaving the ratios of all the components the same. Apply this principle to any size plate or flask.

Table 5 provides some example volumes for commonly used plate and flask sizes. If other sizes of plates or flasks are desired, scale the volume of reagents accordingly. The volumes presented in this table use 10µl of Total Transfection Complex and 0.1µg of DNA per 96-well plate as the reference points.

Table 5. Volumes (per well) for Various Plate/Flask Sizes and ViaFect™ Transfection Reagent:DNA Ratios.

Plate/Flask Size	Volume of Cells in Media (per well)	Total Volume of Transfection Complex (per well)	Amount of DNA (per well)[1]	Amount of ViaFect™ Transfection Reagent (per well) for various ViaFect™ Transfection Reagent:DNA Ratios				
				1.5:1	2:1	3:1	4:1	6:1
96-well plate	100µl	10µl	0.1µg	0.15µl	0.2µl	0.3µl	0.4µl	0.6µl
24-well plate	500µl	50µl	0.5µg	0.75µl	1µl	1.5µl	2µl	3µl
6-well plate	2ml	200µl	2µg	3µl	4µl	6µl	8µl	12µl
T75-flask	15ml	1.5ml	15µg	22.5µl	30µl	45µl	60µl	90µl

[1]DNA amount can be optimized; 1µg/100µl of Opti-MEM® I reduced-serum medium is a recommended starting point.

4.G. Multiplexing for Easy Optimization

The optimal transfection protocol will produce the highest transgene expression with the least possible toxicity. In general, a 96-well plate is sufficient for optimization and provides a relatively easy format for performing cell-based assays. To accurately establish optimal conditions, we find it practical to use a multiplex assay to measure cell viability and reporter gene activity from a single sample in a single well. The procedure to do this using the ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay (Cat.# E7110) is as follows:

1. Follow the instructions in Section 4.E to set up a plate with various ratios and replicates of different transfection conditions. We suggest using a constitutive reporter vector such as pGL4.13[*luc2*/SV40] Vector (Cat.# E6681), pGL4.50[*luc2*/CMV/Hygro] Vector (Cat.#E1310) or pGL4.51[*luc2*/CMV/Neo] Vector (Cat.#E1320).
2. Incubate cells for 24–48 hours.
3. Add 20µl of CellTiter-Fluor™ Reagent (prepared as 10µl of substrate in 2ml of Assay Buffer) to all wells, and mix briefly by orbital shaking (300–500rpm for approximately 30 seconds). Incubate for at least 30 minutes at 37°C.

   Note: Longer incubations may improve assay sensitivity and dynamic range. However, do not incubate longer than 3 hours, and be sure to shield plates from ambient light.
4. Measure resulting fluorescence using a fluorometer or multimode plate reader, such as a GloMax®-Multi+ Detection System (380–400nm$_{Ex}$/505nm$_{Em}$).

   Note: You may need to adjust instrument gains (applied photomultiplier tube energy).
5. Add an equal volume of ONE-Glo™ Luciferase Assay Reagent to the volume of the mixture already in each well (100–120µl per well), incubate for 3 minutes at room temperature and then measure luminescence using a luminometer or multimode plate reader, such as a GloMax®-Multi+ Detection System.
6. Determine conditions that provide the highest luciferase activity and cell viability.

Example Optimization Data

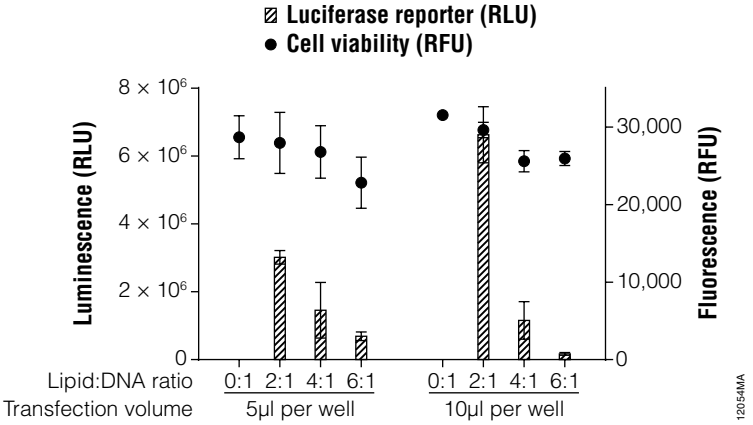

Figure 2. Example of transfection optimization. TF-1 cells were plated in growth media without antibiotics at 30,000 cells per well in a white 96-well assay plate and transfected with a CMV-*luc2* plasmid using various lipid (ViaFect™ Transfection Reagent):DNA ratios. The DNA concentration was held constant at 1µg per 100µl of Opti-MEM® I reduced-serum medium, and the amount of ViaFect™ Transfection Reagent was varied to obtain the indicated ratios. Either 5 or 10µl of transfection complex was then added to cells in the 96-well plate. Twenty-four hours post-transfection, the ONE-Glo™ + Tox Luciferase Assay was performed. Note that 0:1 is the negative control with DNA but no lipid. These results show that, for this particular cell line, a 2:1 ViaFect™ Transfection Reagent:DNA ratio gives optimal results.

5. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Causes and comments
No transfection or low transfection efficiency	Poor-quality DNA. The purified DNA should be transfection quality.
	The A_{260}/A_{280} ratio of the DNA should be 1.7–1.9.
	Suboptimal ratio of ViaFect™ Transfection Reagent:DNA. Optimize the ViaFect™ Transfection Reagent:DNA ratio. Ratios of 2:1 and 3:1 work well for many cell lines, but ratios outside of this range may be optimal for a particular cell type or application.
	Excessive cell death. ViaFect™ Transfection Reagent is one of the more gentle methods of DNA transfection into cells. In the event of cell death, optimize conditions as follows: • Lower the amount of input DNA and ViaFect™ Transfection Reagent while keeping the ratio constant. • Lower the total DNA concentration. • Use higher cell density for the transfection step. • Serum concentration in culture medium was too low for the cell line used. Transfect cells in the presence of serum to determine if transfection in the presence of serum is successful.
	Excessive cell death. Transfected gene products may be toxic.
Variable transfection efficiencies in replicate experiments	Suboptimal growth of cells. • Check that cultures are *Mycoplasma*-free. • Use cultured cells at low passage number.
	Variable cell density. Maintain a consistent cell density at the time of transfection for each experiment.

6. References

1. Southern, P.J. and Berg, P. (1982) Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV40 early region promoter. *J. Mol. Appl. Genet.* **1**, 327–41.
2. Blochlinger, K. and Diggelmann, H. (1984) Hygromycin B phosphotransferase as a selectable marker for DNA transfer experiments with higher eucaryotic cells. *Mol. Cell. Biol.* **4**, 2929–31.

7. Summary of Changes

The following change was made to the 7/17 revision of this document:

1. Updated the storage conditions on page 2.