TECHNICAL BULLETIN

TransFast™ Transfection Reagent

Instructions for Use of Product
E2431

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TB260

TransFast™ Transfection Reagent

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1. Description

Liposomes are one of a number of chemical reagents used to deliver nucleic acids to eukaryotic cells by a nonviral process referred to as transfection. Other types of chemical compounds used for transfection include calcium phosphate and DEAE-dextran (1,2). The term "liposome" refers to lipid bilayers that form colloidal particles in an aqueous medium (3). Liposome reagents specifically designed for transfection applications incorporate synthetic cationic lipids (4), often formulated together with the neutral lipid L-dioleoyl phosphatidylethanolamine (DOPE), which has been demonstrated to enhance the gene transfer ability of certain synthetic cationic lipids (5,6). The TransFast™ Transfection Reagent is comprised of the synthetic cationic lipid, (+)-N,N [bis (2-hydroxyethyl)-N-methyl-N-[2,3-di(tetradecanoyloxy)propyl] ammonium iodide (Figure 1) and the neutral lipid, DOPE. The TransFast™ Reagent is supplied as a dried lipid film that forms multilamellar vesicles upon hydration with water.

Incubation of cationic lipid-containing liposomes and nucleic acids results in quick association and a compaction of the nucleic acid (7,8), presumably from electrostatic interactions between the negatively charged nucleic acid and positively charged head group of the synthetic lipid. The liposome/nucleic acid complex then is presented to the cells that are to be transfected. The liposome complex neutralizes the negative charge of the nucleic acids, allowing closer association of the complex with the negatively charged cell membrane. Entry of the liposome complex into the cell may occur by endocytosis or fusion with the plasma membrane via the lipid moieties of the liposome (9). Once inside the cell, the complexes often become trapped in endosomes and lysosomes (10). Endosomal disruption is facilitated by DOPE, which allows the complexes to escape into the cytoplasm (10). The cytoplasm is the site of action for RNA or antisense oligonucleotides delivered via the liposomes. It is not known precisely how the transfected DNA or liposome/DNA complex gains entry to the nucleus. The best transfection reagent and conditions for a particular cell type must be determined empirically and systematically because inherent properties of the cell influence the success of any specific transfection method.

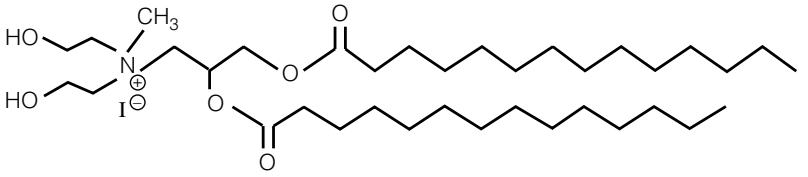

Figure 1. Structure of the synthetic cationic lipid component of the TransFast™ Reagent.

Cationic liposomes designed for transfection, such as the TransFast™ Reagent, are more versatile than many other traditional transfection methods. The advantages include: versatility in the macromolecules that are delivered, in vitro and in vivo applications, ability to more reproducibly transfect cells that are recalcitrant to other methods, and suitability for transient and stable transfection. Several different types of macromolecules including RNA and DNA of all sizes, ranging from oligonucleotides to plasmids and yeast artificial chromosomes, can be delivered to cells using liposomes (11–16). The TransFast™ Transfection Reagent is designed to deliver nucleic acid to eukaryotic cells in vitro and in vivo (17) and performs well with many cell lines. To date, we have found that TransFast™ Reagent performs particularly well for DNA delivery to NIH/3T3, CHO, 293, K562, PC12, Jurkat and insect Sf9 cells, among other cell types. The TransFast™ Reagent combines the advantages of cationic liposome-mediated transfection with the features of speed and ease-of-use.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT. #
TransFast™ Transfection Reagent	**1.2mg**	**E2431**

Contains sufficient reagent to transfect 400µg of DNA (at a 1:1 TransFast™ Reagent:DNA ratio). Includes:

- 1.2mg TransFast™ Transfection Reagent (3 × 0.4mg)
- 2ml Nuclease-Free Water

Storage Conditions: Store dried TransFast™ Reagent (as supplied) at –20°C. Store reconstituted TransFast™ Reagent at –20°C, where it is stable for up to 8 weeks.

Packaging: TransFast™ Reagent is supplied in glass vials under argon gas. Nuclease-Free Water is provided to suspend the lipid film.

3. General Considerations

3.A. Charge Ratio of Transfection Reagent to DNA

For in vitro delivery to cultured cells, the amount of positive charge contributed by the cationic lipid component of the transfection reagent should neutralize or exceed the amount of negative charge contributed by the phosphates on the DNA backbone, resulting in a net neutral or positive charge on the multilamellar vesicles associating with the DNA. Charge ratios of 1:1 to 2:1 TransFast™ Reagent:DNA have worked well with the cell lines listed in Section 1, 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 1.8–1.9). Suspend the ethanol-precipitated DNA in sterile water or TE buffer to 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. For adherent cells, we recommend initially testing 0.25, 0.50, 0.75 and 1µg of DNA per well in a 24-well plate format at a transfection reagent:DNA ratio of 2:1 and 1:1. Increasing the amount of DNA does not necessarily result in higher transfection efficiencies.

3.C. Time

The optimal transfection time depends on the cell line and DNA used. For initial tests, use a 1-hour transfection interval. For many cell lines, a 1-hour transfection interval is optimal; however, for complete optimization, test transfection times from 30 minutes to 4 hours. Monitor cell morphology during the transfection interval, particularly when the cells are maintained in serum-free medium, as some cells lose viability under these conditions. The transfection time with the TransFast™ Reagent can be decreased to as little as 30 minutes with certain cell lines. In addition to saving time, this shortened transfection time may significantly reduce the risk of cell death during the transfection interval.

3.D. Serum

Transfection protocols often require serum-free conditions for optimal performance because serum can interfere with many commercially available transfection reagents. However, the TransFast™ 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. Stable Transfection

TransFast™ Reagent can be used to produce stable transfectants. However, we recommend first optimizing transfection conditions using transient transfection studies prior to applying selective pressure to generate stable transfectants.

4. Recommended Assay Protocol

Figure 2 provides an overview of the transfection procedure. We recommend using a 24-well plate format to optimize transfection conditions for a particular cell type (Figure 3) before initiating any experimental transfections with the TransFast™ Reagent.

Materials to Be Supplied by the User

- cell culture medium with serum (i.e., complete medium, appropriate for the cell type being transfected)
- serum-free cell culture medium
- 24-well or 6-well plates, or 60mm or 100mm cell culture plates

4.A. Plating Cells

Plate cells one day before the transfection experiment so that cells will be approximately 80% confluent on the day of transfection. Some cell lines, such as HeLa cells, exhibit higher toxicity effects when transfected at lower cell densities. As a general guideline, plate 5×10^4 cells per well (24-well plate) or 5.5×10^5 cells (60mm culture dish). Change cell numbers proportionately for different size plates (see Table 1).

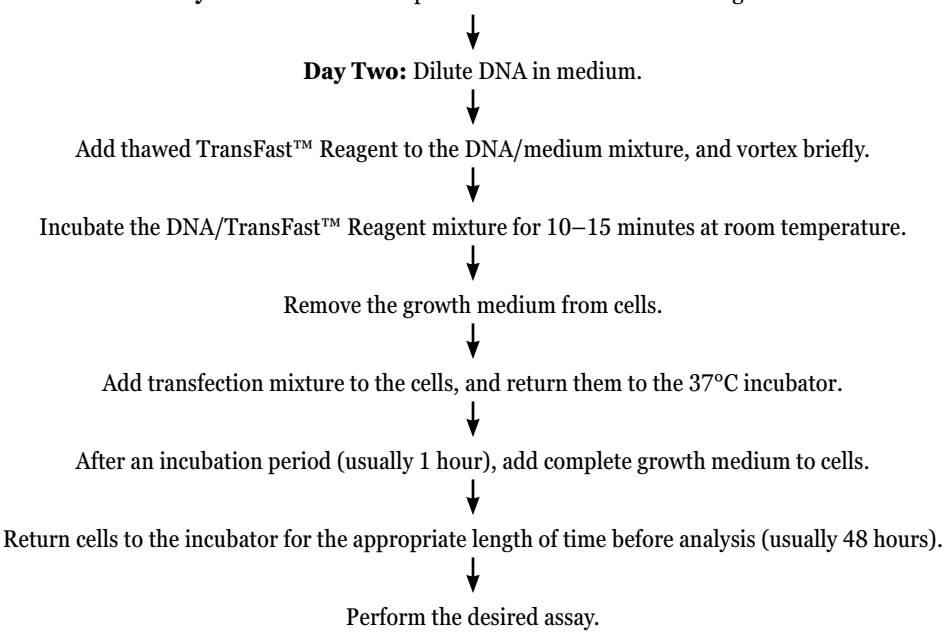

Figure 2. Overview of transfection of adherent cells.

Table 1. Area of Culture Plates for Cell Growth.

Size of Plate	Growth Area (cm^2)[a]	Relative Area[b]
24-well	1.88	1X
96-well	0.32	0.2X
12-well	3.83	2X
6-well	9.4	5X
35mm	8.0	4.2X
60mm	21	11X
100mm	55	29X

[a]This information was calculated for Corning® culture dishes.

[b]Relative area is expressed as a factor of the total growth area of the 24-well plate recommended for optimization studies. To determine the proper plating density, multiply 5×10^4 cells by this factor.

4.B. Preparation of TransFast™ Reagent Stock Solution

1. The **day before transfection**, warm the vial of TransFast™ Reagent to room temperature. Add 400µl of Nuclease-Free Water at room temperature (1mM is the final concentration of the cationic lipid component). The water can be injected into the vial with a syringe, or the entire cap and septum can be removed, water added directly to the vial and the septum then replaced. After adding the Nuclease-Free Water, vortex the reagent vigorously for 10 seconds to suspend the lipid film. Store the vial at –20°C overnight.

   Note: The appearance of the lipid film in the vial may vary. Product performance is not affected.

2. Before each use, thaw at room temperature and vortex the solution. Collect any liquid that may have condensed at the top of the vial or in the vial cap by placing the TransFast™ Reagent vial inside a 50ml centrifuge tube and centrifuging briefly at 300 × *g*. After use, store the remaining stock in the vial at –20°C. The TransFast™ Reagent remains unaffected for 10 freeze-thaw cycles.

   Note: In certain circumstances, the TransFast™ lipid may not suspend completely following reconstitution and freeze-thaw. If this occurs, heat the TransFast™ Reagent to 37°C for 30 minutes after the freeze-thaw cycle to suspend any residual lipid. This heating step will not affect reagent performance. If you need to pool multiple vials for your experiment and a heating step is required, you should combine the vial contents after heating.

4.C. Optimization of Transfection

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 2–3 days after transfection. Promega offers reporter genes and assays for luciferase, chloramphenicol acetyltransferase (CAT) and β-galactosidase (see Section 7).

Initially, we recommend testing various amounts of DNA (0.25, 0.5, 0.75 and 1µg per well) at charge ratios (TransFast™ Reagent to DNA) of 1:1 and 2:1. For this initial optimization, we recommend using serum-free conditions with adherent cells in two 24-well plates per cell line (6 replicates per DNA amount at each charge ratio), and a 1-hour exposure time. Figure 3 outlines a typical optimization matrix.

1. For a 24-well plate, the total volume of medium, DNA and TransFast™ Reagent should be 200µl per well. The volumes given in Tables 2A and 2B were calculated for 7 wells, adequate for 6 replicates for each DNA concentration. In a sterile polypropylene or polystyrene tube, combine the indicated amount of medium (serum-free and prewarmed to 37°C) and plasmid DNA, and vortex. Add the indicated amount of TransFast™ Reagent, and vortex immediately.

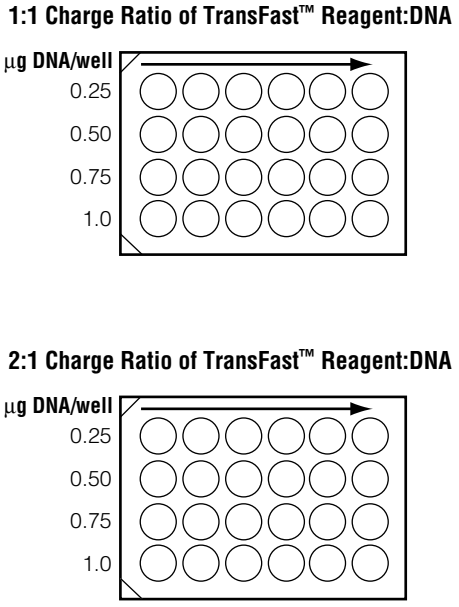

Figure 3. Suggested plating format for the initial optimization of transfection conditions.

Table 2A. Optimization Protocol Using a 1:1 Ratio of TransFast™ Reagent to DNA.

	Amount of DNA per Well			
	0.25µg	**0.5µg**	**0.75µg**	**1µg**
Medium to a final volume of	1,400µl	1,400µl	1,400µl	1,400µl
DNA	1.8µg	3.5µg	5.3µg	7.0µg
TransFast™ Reagent*	5.4µl	10.5µl	15.9µl	21µl

*Volumes given are for use with TransFast™ Reagent suspended in 400µl/vial. The volumes given were calculated for 7 wells, adequate for 6 replicates for each DNA concentration.

Table 2B. Optimization Protocol Using a 2:1 Ratio of TransFast™ Reagent to DNA.

	Amount of DNA per Well			
	0.25µg	**0.5µg**	**0.75µg**	**1µg**
Medium to a final volume of	1,400µl	1,400µl	1,400µl	1,400µl
DNA	1.8µg	3.5µg	5.3µg	7.0µg
TransFast™ Reagent*	10.8µl	21µl	31.8µl	42µl

*Volumes given are for use with TransFast™ Reagent suspended in 400µl/vial. The volumes given were calculated for 7 wells, adequate for 6 replicates for each DNA concentration.

4.C. Optimization of Transfection (continued)

2. Allow the TransFast™ Reagent/DNA mixture to incubate for 10–15 minutes at room temperature. Incubations longer than 30 minutes may result in a lowered transfection efficiency.
3. Carefully remove the growth medium from the cells by aspiration.
4. Briefly vortex the TransFast™ Reagent/DNA mixture. Add the mixture to the cells (200µl per well), and return the plates to the incubator for 1 hour. During the incubation, warm complete medium (cell culture medium containing serum) to 37°C.
5. At the end of the incubation period (1 hour), gently overlay the cells with 1ml of complete medium (prewarmed to 37°C). Do not remove the medium containing the TransFast™ Reagent/DNA mixture. Return the cells to the incubator, and continue the incubation for the appropriate length of time before analysis. For many reporter systems (luciferase, CAT and β-galactosidase) a 48-hour incubation is usually sufficient.
6. Check the transfection efficiency using an assay appropriate for the reporter system.

4.D. Transfection Protocol for Adherent Cells

Note: The TransFast™ 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.

If you have optimized the transfection parameters as described in the previous section, use the empirically determined conditions for your experimental transfections. If you choose not to optimize the transfection parameters, use the general conditions recommended below. Volumes and amounts are given for transfections performed in 60mm plates (values for 100mm plates are given in parentheses). Values for other plates are given in Table 3.

Table 3. Total Volume of Medium, DNA and TransFast™ Reagent for Multiwell Plates.

Plate Size	Total Transfection Volume (per well)	Amount of DNA (per well)
6-well plate	1.0ml	1.25–5µg
12-well plate	400µl	0.5–2µg
24-well plate	200µl	0.25–1µg
96-well plate	40µl	0.05–0.2µg

1. The total volume of medium, DNA and TransFast™ Reagent to add per 60mm plate is 2ml (6ml per 100mm plate). To a sterile tube, add the appropriate amount of medium (prewarmed to 37°C). Add 2.5–10µg of plasmid DNA to the medium (7.5–30µg for a 100mm plate), and vortex. We recommend 5µg of DNA per 60mm plate (15µg per 100mm plate) and a 1:1 lipid:DNA ratio for initial transfection experiments. Add the amount of TransFast™ Reagent indicated in Table 4, and vortex immediately.

Table 4. Relationship Between Volume of TransFast™ Reagent and TransFast™ Reagent:DNA Charge Ratio.

Charge Ratio of TransFast™ Reagent to DNA	Volume of TransFast™ Reagent per µg of DNA*
1:1	3.0µl
2:1	6.0µl
3:1	9.0µl

*Volumes given are for use with TransFast™ Reagent suspended in 400µl/vial.

2. Incubate the TransFast™ Reagent/DNA mixture for 10–15 minutes at room temperature.
3. Remove the growth medium from cells.
4. Briefly vortex the TransFast™ Reagent/DNA mixture. Add 2ml (or 6ml per 100mm plate) of the TransFast™ Reagent/DNA mixture to each plate, and immediately return the cells to the incubator for 1 hour. During the incubation, warm an appropriate volume of complete medium (containing serum) to 37°C.
5. At the end of the incubation period, gently overlay the cells with 4ml (or 12ml per 100mm plate) of complete medium. Do not remove the transfection medium containing the TransFast™ Reagent. Return the cells to the incubator, and continue the incubation for the appropriate length of time before analysis.
6. Check the transfection efficiency using the appropriate assay. For transient transfection, cells are typically harvested 48 hours after transfection.

4.E. Transfection Protocol for Suspension Cells

Note: The TransFast™ 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.

Optimization of transfection parameters can be performed with suspension cells using the following general guidelines: For 1×10^6 cells, test 1, 2, 3 and 4µg DNA at an initial 1:1 charge ratio of TransFast™ Reagent to DNA by incubating for 1 hour in the absence of serum. If desired, additional optimization studies to test the effect of serum and other TransFast™ Reagent to DNA charge ratios can be performed once the optimal amount of DNA is determined.

4.E. Transfection Protocol for Suspension Cells (continued)

1. Suspend the TransFast™ Reagent the day before the transfection, and store at −20°C (see Section 4.B).
2. On the day of transfection, determine the cell density using a hemacytometer, and concentrate enough cells to complete the transfection experiments. As a guideline, 1×10^6 cells per transfection is usually sufficient. Centrifuge the cells for 5 minutes at 300 × *g* in a swinging-bucket rotor. Suspend the cell pellet to a concentration of 2×10^6 cells/ml in serum-free medium. Count the cells, and adjust the volume if necessary.
3. Prepare the TransFast™ Reagent/DNA mixture: To a sterile tube, add the indicated amount of medium (prewarmed to 37°C) and DNA to a total volume of 0.5ml, and vortex. Add the indicated amount of TransFast™ Reagent (Table 5), and vortex immediately.

Table 5. Optimization Protocol for Suspension Cells Using a 1:1 Ratio of TransFast™ Reagent:DNA.

	Amount of DNA per Well			
	1µg	**2µg**	**3µg**	**4µg**
Medium to a final volume of	0.5ml	0.5ml	0.5ml	0.5ml
DNA	1µg	2µg	3µg	4µg
TransFast™ Reagent*	3µl	6µl	9µl	12µl

*Volumes given are for use with TransFast™ Reagent suspended in 400µl/vial.

4. Allow the TransFast™ Reagent and DNA mixture(s) to incubate for 10–15 minutes at room temperature.
5. While the DNA/TransFast™ Reagent mixtures are incubating, dispense 0.5ml of cells (1×10^6 cells) to each well of a 6-well plate.
6. Briefly vortex the TransFast™ Reagent/DNA mixture, and add to the cells (0.5ml/well). Return the cells to the incubator for 1 hour. During the incubation, warm complete medium (containing serum) to 37°C.
7. At the end of the incubation period, add 5ml of prewarmed, complete medium to each well. Return the cells to the incubator, and continue the incubation for the appropriate length of time before analysis. Forty-eight hours is a typical incubation period for both transient transfections and many reporter assays.
8. Check the transfection efficiency using an assay appropriate for the reporter system.

4.F. Protocol for Stable Transfectants

The goal of stable, long-term 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, then plated in selective medium (medium containing the appropriate drug). The use of selective 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 multiwell plates for further propagation in the presence of selective medium.

Several different drug selection markers are commonly used for long-term transfection studies. For example, cells transfected with recombinant vectors containing the bacterial gene for aminoglycoside (e.g., neomycin) phosphotransferase can be selected for stable transformation in the presence of the drug G418 (18). Similarly, expression of the gene for hygromycin B phosphotransferase from the transfected vector will confer resistance to the drug hygromycin B (19).

Before using a particular drug for selection purposes, determine the amount of drug necessary to kill the cells you will be using. This may vary from one cell type to another. Construct a kill curve using varying concentrations of the drug to determine the amount to select resistant clones. The optimal drug concentration is generally the amount 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 gene for drug resistance, such as neomycin phosphotransferase, using the transfection protocols outlined in Section 4.D or 4.E. As a negative control, transfect cells using DNA that does not contain the drug resistance gene.

1. Forty-eight hours post-transfection, trypsinize adherent cells, and plate at several different dilutions (e.g., 1:20, 1:100, 1:200) in selective medium.
2. For the next 14 days, replace the selective medium every 3 to 4 days.
3. During the second week, monitor the cells for distinct “islands” of surviving cells. Cell death should occur in cultures transfected with the negative control plasmid.
4. Transfer individual clones by standard techniques (e.g., using cloning cylinders) to 96-well plates, and continue to maintain cultures in selective medium.

   Note: Alternatively, if single clones are not required, pools of stable transfectants can be maintained.

Calculating Stable Transfection Efficiency

The following procedure may be used to determine the percentage of stable transfectants obtained. Cells subjected to this procedure are fixed and stained and therefore cannot be propagated further.

1. After approximately 14 days of selection in the appropriate drug, monitor the cultures microscopically for the presence of viable cell clones. When distinct “islands” of surviving cells are visible and nontransfected cells have died out, proceed with Step 2.
2. Prepare stain containing 2% methylene blue in 60% methanol.

4.F. Protocol for Stable Transfectants (continued)

3. Remove the growth medium from the cells by aspiration.
4. Add a sufficient volume of stain to the cells to cover the bottom of the dish.
5. Incubate for 5 minutes.
6. Remove the stain, and rinse gently by dipping the plates into a reservoir of deionized water. Shake off excess moisture.
7. Allow the plates to air-dry. The plates can be stored at room temperature.
8. Count the number of colonies, and calculate the percent of transfectants based on the cell dilution and original cell number.

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	Possible Causes and Comments
No transfection or low transfection efficiency	Poor-quality DNA. The DNA should be purified using CsCl gradients or equivalent methods. The A_{260}:A_{280} ratio of the DNA should be 1.8–1.9.
	Suboptimal charge ratio of TransFast™ Reagent to DNA. Optimize the TransFast™ Reagent:DNA charge ratio. Charge ratios of 1:1 and 2:1 work well for many cell lines, but ratios outside this range may be optimal for a particular cell type or application.
	Excessive cell death: • Decrease the time of exposure of cells to the TransFast™ Reagent. • Lower the amount of input DNA and TransFast™ Reagent while holding the charge ratio constant. Increase cell density for the transfection step. • Remove the TransFast™ Reagent/DNA mixture from the cells after the transfection period before adding complete medium. • Test for transfection in the presence of serum. • 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

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7. Related Products

Transfection Reagents

Product	Size	Cat.#
ViaFect™ Transfection Reagent*	0.75ml	E4981
FuGENE® HD Transfection Reagent*	1ml	E2311
FuGENE® 6 Transfection Reagent*	1ml	E2691
ProFection® Mammalian Transfection Systems—Calcium Phosphate	40 reactions	E1200

*Available in additional sizes.

pGL4 Luciferase Reporter Vectors

Please visit **www.promega.com** to see a complete listing of our reporter vectors.

Vector	Multiple Cloning Region	Reporter Gene	Protein Degradation Sequence	Reporter Gene Promoter	Mammalian Selectable Marker	Cat.#
pGL4.10[*luc2*]	Yes	*luc2*[A]	No	No	No	E6651
pGL4.11[*luc2P*]	Yes	“	hPEST	No	No	E6661
pGL4.12[*luc2CP*]	Yes	“	hCL1-hPEST	No	No	E6671
pGL4.13[*luc2*/SV40]	No	“	No	SV40	No	E6681
pGL4.14[*luc2*/Hygro]	Yes	“	No	No	Hygro	E6691
pGL4.15[*luc2P*/Hygro]	Yes	“	hPEST	No	Hygro	E6701
pGL4.16[*luc2CP*/Hygro]	Yes	“	hCL1-hPEST	No	Hygro	E6711
pGL4.17[*luc2*/Neo]	Yes	“	No	No	Neo	E6721
pGL4.18[*luc2P*/Neo]	Yes	“	hPEST	No	Neo	E6731
pGL4.19[*luc2CP*/Neo]	Yes	“	hCL1-hPEST	No	Neo	E6741
pGL4.20[*luc2*/Puro]	Yes	“	No	No	Puro	E6751
pGL4.21[*luc2P*/Puro]	Yes	“	hPEST	No	Puro	E6761
pGL4.22[*luc2CP*/Puro]	Yes	“	hCL1-hPEST	No	Puro	E6771
pGL4.70[*hRluc*]	Yes	*hRluc*[B]	No	No	No	E6881
pGL4.71[*hRlucP*]	Yes	“	hPEST	No	No	E6891
pGL4.72[*hRlucCP*]	Yes	“	hCL1-hPEST	No	No	E6901
pGL4.73[*hRluc*/SV40]	No	“	No	SV40	No	E6911
pGL4.74[*hRluc*/TK]	No	“	No	HSV-TK	No	E6921
pGL4.75[*hRluc*/CMV]	No	“	No	CMV	No	E6931
pGL4.76[*hRluc*/Hygro]	Yes	“	No	No	Hygro	E6941

Vector	Multiple Cloning Region	Reporter Gene	Protein Degradation Sequence	Reporter Gene Promoter	Mammalian Selectable Marker	Cat.#
pGL4.77[*hRlucP*/Hygro]	Yes	“	hPEST	No	Hygro	E6951
pGL4.78[*hRlucCP*/Hygro]	Yes	“	hCL1-hPEST	No	Hygro	E6961
pGL4.79[*hRluc*/Neo]	Yes	“	No	No	Neo	E6971
pGL4.80[*hRlucP*/Neo]	Yes	“	hPEST	No	Neo	E6981
pGL4.81[*hRlucCP*/Neo]	Yes	“	hCL1-hPEST	No	Neo	E6991
pGL4.82[*hRluc*/Puro]	Yes	“	No	No	Puro	E7501
pGL4.83[*hRlucP*/Puro]	Yes	“	hPEST	No	Puro	E7511
pGL4.84[*hRlucCP*/Puro]	Yes	“	hCL1-hPEST	No	Puro	E7521

[A]*luc2* = synthetic firefly luciferase gene.

[B]*hRluc* = synthetic *Renilla* luciferase gene.

Luminometers

Product	Cat.#
GloMax® Discover System	GM3000

Plasmid DNA Purification Systems

Product	Size	Cat.#
PureYield™ Plasmid Midiprep System	25 preps*	A2492

*Available in additional sizes.

8. Summary of Changes

The following changes were made to the 9/15 revision of this document:

1. The patent information was updated to remove expired statements.
2. The document design was updated.
3. Related products were updated.