

TECHNICAL MANUAL

FuGENE[®] 6 Transfection Reagent

Instructions for Use of Products
E2691, E2692 and E2693

FuGENE® 6 Transfection Reagent

All technical literature is available at: www.promega.com/protocols/
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1. Description	2
2. Product Components and Storage Conditions	2
3. General Considerations.....	2
3.A. Ratio of Transfection Reagent to DNA.....	3
3.B. DNA	3
3.C. Time	3
3.D. Serum	3
3.E. Antibiotics	3
3.F. Stable Transfection	3
4. Recommended Protocol	4
4.A. Plating Cells.....	5
4.B. Preparing the FuGENE® 6 Transfection Reagent.....	5
4.C. General Transfection Protocol	6
4.D. Protocol for Stable Transfection	7
4.E. Transfection Optimization	8
4.F. Multiplexing for Easy Optimization.....	9
5. Troubleshooting	10
6. References	10
7. Related Products	11
8. Summary of Changes	14

1. Description

FuGENE® 6 Transfection Reagent^(a) is a nonliposomal reagent that transfects DNA into a wide variety of cell lines with high efficiency and low toxicity. 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.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT. #
FuGENE® 6 Transfection Reagent	0.5ml	E2693
	1ml	E2691
	5 × 1ml	E2692

The 1ml size contains sufficient reagent to transfect 333µg of DNA. This is equivalent to over 3,300 wells in 96-well plates at a 3:1 FuGENE® 6 Transfection Reagent:DNA ratio (0.3µl reagent:100ng DNA per well). The actual number of transfections will vary with reagent:DNA ratio, transfection volume and cell type.

Storage Conditions: Store FuGENE® 6 Transfection Reagent at +2°C to +10°C. Close lid tightly after use. Do not freeze or store below 0°C.

Formulation and Packaging: FuGENE® 6 Transfection Reagent is a proprietary mixture of lipids and other components in 80% ethanol, filtered through a 0.1µm filter and supplied in glass vials.

Special Handling: Allow FuGENE® 6 Transfection Reagent to reach room temperature, and mix briefly by inverting or vortexing prior to use.

Use a standard 24-well tissue culture plate as a rack for FuGENE® 6 Transfection Reagent. Do not dispense FuGENE® 6 Transfection Reagent into aliquots from the original glass vials. Minimize contact of undiluted FuGENE® 6 Transfection Reagent with plastic surfaces. Do not use siliconized pipette tips or tubes. Always dilute FuGENE® 6 Transfection Reagent directly into medium without contacting the side of the tube.

3. General Considerations

Successful transfection involves optimizing the FuGENE® 6 Transfection Reagent:DNA ratio, amount of DNA used, complexing time, 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 1–2 days after transfection. We offer 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 to DNA

For successful transfection of DNA into cultured cells, the ratio of FuGENE® 6 Transfection Reagent:DNA must be optimized. Ratios of 1.5:1 to 3:1 FuGENE® 6 Transfection Reagent:DNA work well with many cell lines, but ratios outside of this range (up to 6:1) 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. For adherent cells, we recommend initially testing 100ng of DNA per well in a 96-well plate format at FuGENE® 6 Transfection Reagent: DNA ratios of 1.5:1, 3:1 and 6:1. Increasing the amount of DNA does not necessarily result in higher transfection efficiencies.

3.C. Time

The time required to form the FuGENE® 6 Transfection Reagent/DNA complex is 15 minutes at room temperature. Incubate transfected cells for 24–48 hours before assaying to allow time to express the 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. FuGENE® 6 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

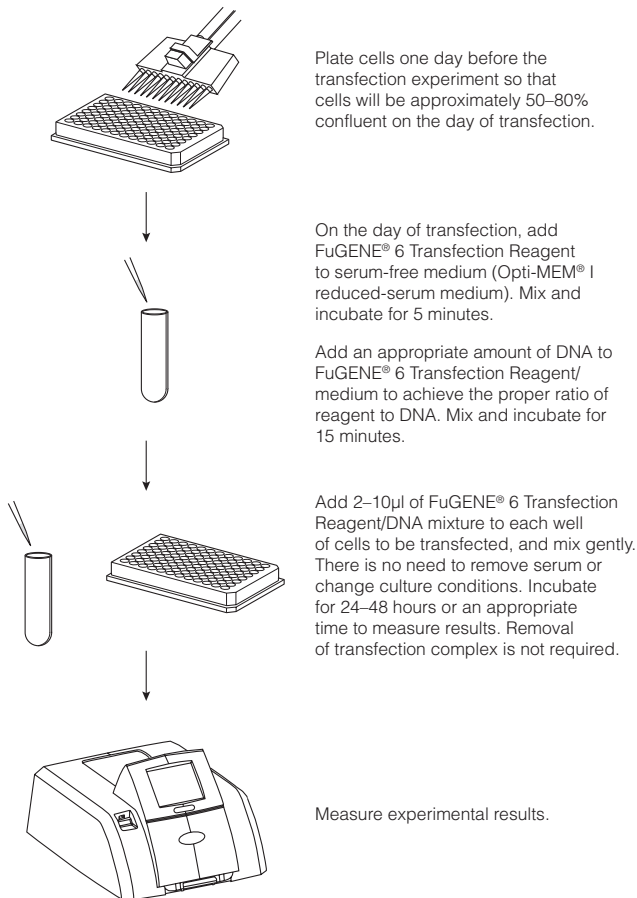
Antibiotics can be used during the culture of cell lines. The presence of antibiotics during transfection may adversely affect transfection efficiency and the overall health of transfected cells. We do not recommend using antibiotics in the transfection medium unless previously tested in the cell type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

3.F. Stable Transfection

FuGENE® 6 Transfection Reagent can be used to produce stable transfectants. However, we recommend optimizing transfection conditions using transient transfection studies prior to applying selective pressure to generate 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.



97291A

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 (Opti-MEM® I reduced-serum medium)
- 96-well or other culture plates
- 24-well culture plate to serve as a rack for FuGENE® 6 Transfection Reagent

4.A. Plating Cells

Plate adherent cells one day before transfection so that cells are approximately 50–80% confluent on the day of transfection. Suspension cells can be plated the day of transfection. As a general guideline, plate $1-2 \times 10^4$ adherent cells or 2×10^4 to 1×10^5 suspension cells in 100µl per well of a 96-well plate. Adjust cell numbers proportionately for different size plates (see Table 1). To prepare cells, collect enough cells to complete the transfection experiment, and centrifuge for 5 minutes at $300 \times g$ in a swinging-bucket rotor. Suspend the cell pellet to an appropriate concentration in medium, then plate.

Table 1. 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

¹This information was calculated for Corning® culture dishes.

²Relative 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 $1-2 \times 10^4$ cells by this factor.

4.B. Preparing the FuGENE® 6 Transfection Reagent

1. Before use, allow the vial of FuGENE® 6 Transfection Reagent to reach room temperature.
2. Mix by inverting or vortexing 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 medium, DNA and FuGENE® 6 Transfection Reagent to add per well of a 96-well plate is 2–10µl (Table 2). To a sterile polystyrene tube or well of a U- or V-bottom plate, add 90–98µl of medium prewarmed to room temperature so that the total volume of medium plus DNA, which is added in Step 2, is 100µl. For a 3:1 FuGENE® 6 Transfection Reagent:DNA ratio, add 6µl of FuGENE® 6 Transfection Reagent to the medium, and mix immediately.


 Add FuGENE® 6 Transfection Reagent directly to medium. Do not allow undiluted FuGENE® 6 Transfection Reagent to contact the sides of the tube or U- or V-bottom plate. Incubate the FuGENE® 6 Transfection Reagent/medium mixture for 5 minutes at room temperature.

Table 2. Total Volume of Medium, DNA and FuGENE® 6 Transfection Reagent for 96-Well Plates at a 3:1 FuGENE® 6 Transfection Reagent:DNA Ratio.

Plate Size	Total Transfection Volume (Per Well) ¹	Amount of FuGENE® 6 Reagent (Per Well) ¹	Amount of DNA (Per Well) ¹
96-well	2–10µl	0.15–0.6µl	0.04–0.2µg

¹See Table 1 for multiplication factors to scale up for larger wells or plates. See Table 3 for FuGENE® 6 Transfection Reagent volumes at different reagent: DNA ratios.

2. Add 2µg of plasmid DNA (0.2–1µg/µl) to the FuGENE® 6 Transfection Reagent/medium, and mix immediately. Incubate the FuGENE® 6 Transfection Reagent/DNA mixture for 15 minutes at room temperature.
Note: Incubations longer than 45 minutes may adversely affect transfections.
3. Add 2–10µl of the FuGENE® 6 Transfection Reagent/DNA mixture to each well of a 96-well plate containing 100µl of cells in growth medium. We suggest 5µl of mixture as a starting point. Mix 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, then plated in 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 then are trypsinized and transferred to flasks for further propagation or to multiwell plates for limited dilution cloning in the presence of selective 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 (e.g., neomycin) phosphotransferase can be selected for stable transformation 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). We offer vectors conferring resistance to G-418, hygromycin B or puromycin.

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 needed 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 using the transfection protocols outlined in Sections 4.C and 4.E.

Optional: As a selection-drug-negative control, transfect cells using DNA that does not contain the drug-resistance gene.

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 to 4 days.
3. During the second week, monitor cells for distinct colonies of surviving cells. Complete cell death should occur in cultures 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.

4.E. Transfection Optimization

We strongly recommend that you optimize transfection conditions for each cell line. For this initial optimization, we recommend using 50–200ng of DNA per well at various ratios of FuGENE® 6 Transfection Reagent to DNA (Table 3). We recommend using standard growth conditions with cells in 96-well plates. For a detailed discussion on optimization and a spreadsheet to aid data analysis, please visit: www.promega.com/resources/tools/transfection-assistant/


Table 3. Optimization Protocol Using Varying Ratios of FuGENE® 6 Transfection Reagent to DNA.

	Ratio of FuGENE® 6 Transfection Reagent to DNA			
	6:1	4:1	3:1	1.5:1
Medium to a final volume of ¹	100µl	100µl	100µl	100µl
DNA amount	2µg	2µg	2µg	2µg
Volume of FuGENE® 6 Transfection Reagent ¹	12µl	8µl	6µl	3µl

¹The volumes were calculated for 20 wells (5µl/well) of a 96-well plate for each ratio.

- For a 96-well plate, the total volume of medium and cells per well prior to transfection should be 100µl. The volume of FuGENE® 6 Transfection Reagent/DNA complex added should be optimized; 2–10µl 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 1).

In a sterile polystyrene tube or well of a U- or V-bottom plate, combine the indicated amount of medium (prewarmed to room temperature) and FuGENE® 6 Transfection Reagent. Mix immediately, and incubate at room temperature for 5 minutes.

-  Add FuGENE® 6 Transfection Reagent directly to medium; do not allow undiluted FuGENE® 6 Transfection Reagent to contact the sides of the tube or plate.
- Add the indicated amount of plasmid DNA, and mix immediately. Allow the FuGENE® 6 Transfection Reagent/DNA complex to incubate at room temperature for 15 minutes.
Note: Incubations longer than 45 minutes may adversely affect transfections.
- Add 2–10µl of complex per well to a 96-well plate containing 100µl of cells in growth medium. Mix by pipetting or using a plate shaker for 10–30 seconds. Return plates to the incubator. For many reporter systems (luciferase, CAT, β-galactosidase, etc.) a 24- to 48-hour incubation is sufficient.
- Check the transfection efficiency using an assay appropriate for the reporter system. To multiplex two reporter assays or one reporter assay with cell viability or toxicity measurement, see Section 4.F.

4.F. Multiplexing for Easy Optimization

The optimal transfection protocol will give the highest transgene expression with the lowest possible toxicity. In general a 96-well plate provides a sufficient number of sample wells to perform 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 for each sample in a single well. The procedure to do this using the CellTiter-Fluor™ Cell Viability Assay (Cat.# G6080) and ONE-Glo™ Luciferase Assay System (Cat.# E6110) is as follows:

1. Follow the instructions in Section 4.E to create a plate with titrations and replicates of different transfection conditions. Use a plasmid DNA encoding a constitutive reporter gene such as the pGL4.13[*uc2*/SV40] Vector (Cat.# E6681).
2. Culture 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. 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 with Instinct™ Software (380–400_{Ex}/505_{Em}, see Section 7).
Note: You may need to adjust instrument gains (applied photomultiplier tube energy).
5. Add an equal volume of ONE-Glo™ Luciferase Assay Reagent prepared as described in Technical Manual #TM292 to wells (100–120µl per well), incubate for 3 minutes, then measure luminescence using a luminometer or multimode plate reader, such as a GloMax®-Multi+ Detection System with Instinct™ Software.
6. Determine the condition that provides the highest luciferase activity and viability. To assist data analysis, a spreadsheet that plots data from this experiment is available at:
www.promega.com/resources/tools/transfection-assistant/
Note that the instructions and plate layout in the spreadsheet need to be strictly followed.

5. Troubleshooting

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

Symptoms	Causes and Comments
No transfection or low transfection efficiency	<p>Poor-quality DNA. The purified DNA should be transfection-quality. We routinely use the PureYield™ Plasmid Midiprep or Maxiprep Systems to generate transfection-quality DNA for many cell lines. The A_{260}/A_{280} ratio of the DNA should be 1.7–1.9.</p> <p>Suboptimal ratio of FuGENE® 6 Transfection Reagent to DNA. Optimize the FuGENE® 6 Transfection Reagent:DNA ratio. Ratios of 3:1 and 1.5:1 work well for many cell lines, but ratios outside this range may be optimal for a particular cell type or application.</p> <p>Excessive cell death. FuGENE® 6 Transfection Reagent is one of the more gentle methods of DNA transfection into cells. In the event of cell death, optimize conditions as follows:</p> <ul style="list-style-type: none"> • Lower the amount of input DNA and FuGENE® 6 Transfection Reagent while holding the ratio constant. • Increase 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. <p>Excessive cell death. Transfected gene products may be toxic.</p>
Variable transfection efficiencies in replicate experiments	<p>Suboptimal growth of cells.</p> <ul style="list-style-type: none"> • Check that cultures are <i>Mycoplasma</i>-free. • Use cultured cells at low passage number. <p>Variable cell density. Maintain a consistent cell density at the time of transfection for each experiment.</p>

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. Related Products

Transfection Reagent

Product	Size	Cat.#
FuGENE® HD Transfection Reagent	1ml	E2311
	5 × 1ml	E2312

pGL4 Luciferase Reporter Vectors

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

Vector	Multiple Cloning Region	Reporter Gene	Protein Degradation Sequence	Reporter Gene Promoter	Mammalian Selectable Marker	Cat.#
pGL4.10[<i>luc2</i>]	Yes	<i>luc2</i> ^A	No	No	No	E6651
pGL4.11[<i>luc2P</i>]	Yes	"	hPEST	No	No	E6661
pGL4.12[<i>luc2CP</i>]	Yes	"	hCL1-hPEST	No	No	E6671
pGL4.13[<i>luc2</i> /SV40]	No	"	No	SV40	No	E6681
pGL4.14[<i>luc2</i> /Hygro]	Yes	"	No	No	Hygro	E6691
pGL4.15[<i>luc2P</i> /Hygro]	Yes	"	hPEST	No	Hygro	E6701
pGL4.16[<i>luc2CP</i> /Hygro]	Yes	"	hCL1-hPEST	No	Hygro	E6711
pGL4.17[<i>luc2</i> /Neo]	Yes	"	No	No	Neo	E6721
pGL4.18[<i>luc2P</i> /Neo]	Yes	"	hPEST	No	Neo	E6731
pGL4.19[<i>luc2CP</i> /Neo]	Yes	"	hCL1-hPEST	No	Neo	E6741
pGL4.20[<i>luc2</i> /Puro]	Yes	"	No	No	Puro	E6751
pGL4.21[<i>luc2P</i> /Puro]	Yes	"	hPEST	No	Puro	E6761
pGL4.22[<i>luc2CP</i> /Puro]	Yes	"	hCL1-hPEST	No	Puro	E6771
pGL4.23[<i>luc2</i> /minP]	Yes	"	No	minP	No	E8411
pGL4.24[<i>luc2P</i> /minP]	Yes	"	hPEST	minP	No	E8421
pGL4.25[<i>luc2CP</i> /minP]	Yes	"	hCL1-hPEST	minP	No	E8431
pGL4.26[<i>luc2</i> /minP/Hygro]	Yes	"	No	minP	Hygro	E8441

pGL4 Luciferase Reporter Vectors (continued)

Vector	Multiple Cloning Region	Reporter Gene	Protein Degradation Sequence	Reporter Gene Promoter	Mammalian Selectable Marker	Cat. #
pGL4.27[<i>luc2P</i> /minP/Hygro]	Yes	<i>luc2</i> ^A	hPEST	minP	Hygro	E8451
pGL4.28[<i>luc2CP</i> /minP/Hygro]	Yes	"	hCL1-hPEST	minP	Hygro	E8461
pGL4.29[<i>luc2P</i> /CRE/Hygro]	No	"	hPEST	CRE	Hygro	E8471
pGL4.30[<i>luc2P</i> /NFAT-RE/Hygro]	No	"	hPEST	NFAT-RE	Hygro	E8481
pGL4.31[<i>luc2P</i> /GAL4UAS/Hygro]	No	"	hPEST	GAL4 UAS	Hygro	C9351
pGL4.32[<i>luc2P</i> /NF-κB-RE/Hygro]	No	"	hPEST	NF-κB-RE	Hygro	E8491
pGL4.33[<i>luc2P</i> /SRE/Hygro]	No	"	hPEST	SRE	Hygro	E1340
pGL4.34[<i>luc2P</i> /SRF-RE/Hygro]	No	"	hPEST	SRF-RE	Hygro	E1350
pGL4.36[<i>luc2P</i> /MMTV/Hygro]	No	"	hPEST	MMTV	Hygro	E1360
pGL4.50[<i>luc2</i> /CMV/Hygro]	No	"	No	CMV	Hygro	E1310
pGL4.51[<i>luc2</i> /CMV/Neo]	No	"	No	CMV	Neo	E1320
pGL4.70[<i>hRluc</i>]	Yes	<i>hRluc</i> ^B	No	No	No	E6881
pGL4.71[<i>hRlucP</i>]	Yes	"	hPEST	No	No	E6891
pGL4.72[<i>hRlucCP</i>]	Yes	"	hCL1-hPEST	No	No	E6901
pGL4.73[<i>hRluc</i> /SV40]	No	"	No	SV40	No	E6911
pGL4.74[<i>hRluc</i> /TK]	No	"	No	HSV-TK	No	E6921
pGL4.75[<i>hRluc</i> /CMV]	No	"	No	CMV	No	E6931

^A*luc2* = synthetic firefly luciferase gene.

^B*hRluc* = synthetic *Renilla* luciferase gene.

Luminometers

For a complete list of Promega luminometers visit:

www.promega.com/products/microplate-readers-fluorometers-luminometers/microplate-luminometers/

Cell Viability Assays

Product	Size	Cat.#
CellTiter-Glo® Luminescent Cell Viability Assay	10ml	G7570
CytoTox-Glo™ Cytotoxicity Assay	10ml	G9290
CellTiter-Fluor™ Cell Viability Assay	10ml	G6080

Additional sizes available.

Plasmid DNA Purification Systems

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

Additional sizes available.



8. Summary of Changes

The following changes were made to the 8/25 revision of this document:

1. Updated the cover and fonts.
2. Made minor miscellaneous text edits, including updating storage conditions and two urls to point to the Transfection Assistant and another url pointing to a complete Luminometer listing.
3. Removed discontinued vectors and luminometers from Section 7, Related Products.

^(a)**FuGENE® 6 is sold with a Limited Use Label License (LULL).** You shall use this Product in accordance with all applicable laws, rules and regulations. You may not attempt to reverse engineer this Product. If you are not willing to accept these conditions, the distributor from whom the Product was purchased will accept the return of the unopened Product and provide you with a full refund. However, in the event this Product is opened, you agree to be bound by the conditions of this limited use statement.

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