



TECHNICAL MANUAL

# FuGENE<sup>®</sup> SI Transfection Reagent

Instructions for Use of Products  
E9311 and E9312

# FuGENE<sup>®</sup> SI Transfection Reagent

All technical literature is available at: [www.promega.com/protocols/](http://www.promega.com/protocols/)  
 Visit the web site to verify that you are using the most current version of this Technical Manual.  
 E-mail Promega Technical Services if you have questions on use of this system: [techserv@promega.com](mailto:techserv@promega.com)

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## 1. Description

The FuGENE® SI Transfection Reagent<sup>(a)</sup> is a lipid-based transfection reagent designed to transfect siRNA, miRNA and other small RNA molecules into a wide variety of eukaryotic cell lines, including insect cells. This reagent can be used to transfect common and difficult-to-transfect cell lines with high efficiency and low toxicity, requiring fewer cells and eliminating the need to change medium after transfection.

## 2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
FuGENE® SI Transfection Reagent	1ml	E9311
	5 × 1ml	E9312

**Storage Conditions:** Store FuGENE® SI Transfection Reagent at +2°C to +10°C. Close lid tightly after use.

**Note:** The 1ml size contains sufficient FuGENE® SI Transfection Reagent to perform up to 3,333 transfections in a 96-well plate with 0.3µl of FuGENE® SI Transfection Reagent and 1pmol of siRNA molecules per well. The actual number of transfections will vary with reagent volume per transfection.

**Formulation and Packaging:** FuGENE® SI Transfection Reagent is a 100% synthetic, proprietary mixture of lipids and other components in 70% ethanol. The transfection reagent is sterile filtered and supplied in glass vials. The FuGENE® SI Transfection Reagent does not contain any human or animal origin ingredients.

**Special Handling:** Equilibrate FuGENE® SI Transfection Reagent to room temperature and mix briefly by inverting or vortexing prior to use. No precipitate should be visible. Do not dispense FuGENE® SI Transfection Reagent into aliquots from the original glass vials. Avoid directly contacting plastic tube surfaces with undiluted FuGENE® SI Transfection Reagent. Always dilute FuGENE® SI Transfection Reagent directly into serum-free transfection medium without touching the side of the tube.

### 3. General Considerations

Transfection using the FuGENE® SI Transfection Reagent requires minimal optimization for many cell types. However, for best results, optimize the transfection conditions for your cell line of interest. Successful transfection involves optimizing the FuGENE® SI Transfection Reagent volume, RNA amount and transfection complex volume per transfection, and complex incubation time. Transfection efficiency also can depend on the gene target, transfection time, cell type and cell culture conditions.

When optimizing transfection conditions for your cell line, some changes in transfection conditions can affect transfection efficiency significantly while variations in other transfection conditions have little or no effect on transfection efficiency such that there is a performance plateau. In our experience, performance plateaus tend to be relatively broad. We recommend choosing transfection conditions in the middle of any such plateaus. Avoid conditions at the edge of performance plateaus because small procedural differences can cause large differences in transfection efficiency.

We recommend using a 96-well plate format to optimize transfection conditions for your cell line of interest. For a detailed optimization protocol, see Section 4.E.

#### 3.A. Transfection Reagent Volume

For optimal transfection of small RNA into cultured cells, you need to optimize the FuGENE® SI Transfection Reagent volume. The protocols in this technical manual use 0.3µl of FuGENE® SI Transfection Reagent as a starting point for each transfection in a 96-well plate. Other volumes may be optimal for other cell types. We recommend testing 0.15–0.6µl of FuGENE® SI Transfection Reagent per transfection in a 96-well plate. The recommended starting volumes for other culture vessels are listed in Table 1.

#### 3.B. RNA Amount

The RNA amount per transfection also must be optimized. We recommend using 10nM RNA as a starting point; this equates to 1pmol per transfection of 100µl of cells in a 96 well plate. We recommend testing 1–100nM (0.1–10.0pmol) of RNA per transfection in a 96-well plate to determine the optimal RNA amount. The recommended starting amounts for other culture vessels are listed in Table 1.

#### 3.C. Transfection Complex Volume

We recommend optimizing the volume of FuGENE® SI Transfection Reagent-RNA complex added to the cells. The protocols in this technical manual use 10µl of transfection complex per well of a 96-well plate, but other volumes may be optimal, depending on the transfection conditions. We recommend evaluating transfection efficiency using 2–10µl of transfection complex per well of a 96-well plate.

#### 3.D. Transfection Complex Incubation Time

We recommend optimizing the incubation time to form the FuGENE® SI Transfection Reagent-RNA complex. The standard time is 5 minutes at 22°C, but you may wish to evaluate incubation times of up to 15 minutes for your cell line. To evaluate additional times, add the transfection complex to cells at different time points after combining reagent and RNA (e.g., 10 minutes and 15 minutes). We do not recommend incubation times longer than 15 minutes.

### **3.E. Serum**

Transfection protocols often require serum-free conditions for optimal performance because serum can interfere with many commercially available transfection reagents. The FuGENE® SI Transfection Reagent can be used in transfection protocols with serum present to transfect cell types that require continuous exposure to serum, such as primary cell cultures. However, the reagent-RNA transfection complex needs to be formed in the absence of serum.

### **3.F. Cell Handling and Culture Conditions**

The optimal cell number can vary, depending on the cell line, transfection protocol used, cell culture conditions and other factors. Seed cell cultures at the proper density and observe cultured cells so that the monolayer is at the desired degree of confluency when transfected. The optimal degree of confluency at transfection must be determined empirically. We recommend 25–50% confluency on the day of transfection as a starting point. If you desire cells that are less confluent at the time of the assay (24–72 hours after transfection), try seeding cells so that they are <25% confluent on the day of transfection and use less transfection complex.

Table 1 in Section 4.A lists suggested seeding densities for various culture vessels for the traditional transfection protocol where cells are plated on the day before transfection. The rapid and reverse protocols typically require twice the cell density as the traditional transfection protocol. Adjust cell numbers proportionately for different sized culture vessels (see Table 2).

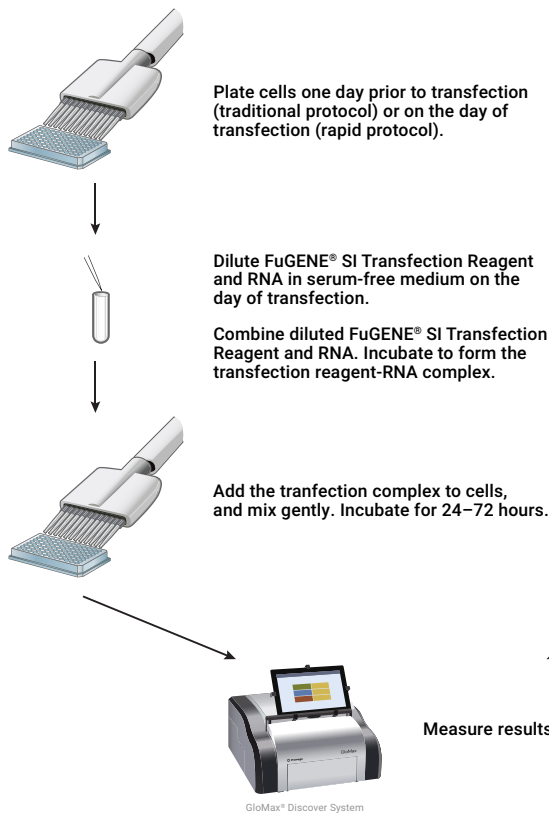
Transfection efficiency can depend on cell culture conditions including cell type, passage level, passage history, seeding density, seeding protocol, growth medium, serum concentration, serum lot, culture vessel and laboratory practices. To minimize intra- and interexperimental variance in transfection efficiency, use cells that are proliferating well (in log-growth phase), passaged regularly and plated at a consistent density.

Antimicrobial agents such as antibiotics and fungicides are often used during cell culture. However, the presence of these antimicrobial agents during transfection can adversely affect transfection efficiency of some cell lines and the overall health of transfected cells. We do not recommend using antimicrobial agents in the transfection medium unless previously tested in the cell type being transfected.

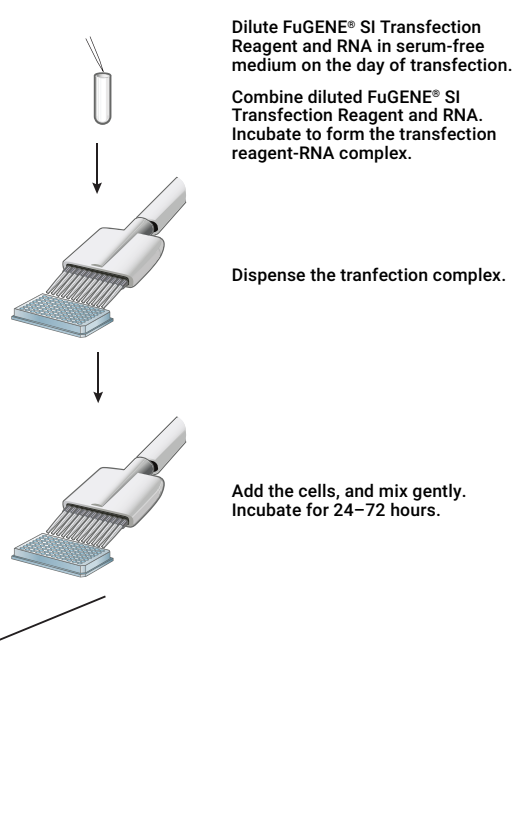
## 4. Protocols

Figure 1, Panel A provides an overview of the traditional and rapid transfection protocols where the FuGENE® SI Transfection Reagent-RNA complex is added to cells in the assay plate. In Figure 1, Panel B, the reverse transfection protocol differs in that cells are added to transfection complex in the assay plate.

### A. Traditional and Rapid Transfection Protocol



### B. Reverse Transfection Protocol



**Figure 1. Overview of the FuGENE® SI Transfection Reagent transfection protocols.**

#### 4. Protocols (continued)

These protocols describe transfecting cells in wells of a 96-well plate. Table 1 lists the appropriate volumes of FuGENE® SI Transfection Reagent, FuGENE® SI Transfection Reagent-RNA complex and cells in growth medium for other culture vessels.

We recommend including the following controls in your transfection experiment:

<b>Cells control</b>	Medium alone (e.g., 10µl of serum-free medium)
<b>RNA control</b>	RNA without FuGENE® SI Transfection Reagent (e.g., 1pmol of RNA in a 10µl volume)
<b>Reagent control</b>	FuGENE® SI Transfection Reagent without RNA (e.g., 0.3µl of reagent in 10µl of serum-free medium)
<b>Positive control</b>	An RNA known to have a measurable effect in your cell line of interest.
<b>Negative control</b>	An RNA that has no effect on your cell line.

#### Materials to Be Supplied by the User

- sterile cell culture medium with serum as appropriate for the cell type being transfected (growth medium)
- sterile serum-free cell culture medium without antimicrobial agents (transfection medium)
- 96-well plate or other culture vessel
- siRNA, miRNA or other small RNA

#### 4.A. Preparing Cells

Seed cell cultures at the proper density and observe cultured cells so that the monolayer is at the desired degree of confluency at the time of transfection. See Section 3.F for recommendations. Table 1 lists suggested seeding densities for various culture vessels for the traditional transfection protocol where cells are plated on the day before transfection. The rapid and reverse protocols typically require twice the cell density as the traditional transfection protocol. The optimal seeding density depends on selected cell line, cell culture conditions and other factors; see Section 3. Adjust cell numbers proportionately for different sized culture vessels (see Tables 1 and 2).

To prepare cells for transfection, choose one of the following methods described below. Be sure to prepare sufficient cells for the control wells.

**Traditional Protocol:** The day before transfection, collect enough cells for your desired seeding density and centrifuge for 5 minutes at 300 × *g* in a swinging-bucket rotor. Adjust the cell concentration to the desired seeding density with growth medium, and transfer cells to the desired culture dish or plate. Incubate cells overnight using incubation conditions appropriate for the cell line being transfected. Proceed to Section 4.B.

**Rapid and Reverse Protocols:** On the day of transfection, collect enough cells to complete the transfection experiment (typically twice as many cells than would be used in the traditional protocol) and centrifuge for 5 minutes at 300 × *g* in a swinging-bucket rotor. Add growth medium to adjust the cell concentration to twice the cell density used for the traditional transfection protocol. Proceed to Section 4.B.

**Table 1. Guidelines for Preparing FuGENE® SI Transfection Reagent-RNA Complex for Various Culture Vessel Sizes and Seeding Densities.** These amounts are provided as starting points. For best results, we recommend optimizing these parameters for your cell type, cell culture conditions and application.

Culture Vessel	Suggested Volume of Transfection Complex Per Well	Suggested Volume of Growth Medium	Suggested Seeding Density (Cells/Well <sup>1</sup> )		Suggested Amount of siRNA Per Transfection (pmol)	Suggested Volume of FuGENE® SI Transfection Reagent Per Transfection
			Low	High		
96-well plate (1 well)	10µl	100µl	5,000	30,000	1	0.3µl
24-well plate (1 well)	50µl	500µl	25,000	150,000	5	1.5µl
12-well plate (1 well)	100µl	1.0ml	50,000	300,000	10	3.0µl
35mm dish or six-well plate (1 well)	250µl	2.5ml	125,000	750,000	25	7.5µl
60mm dish	500µl	5.0ml	250,000	1,500,000	50	15µl
10cm dish	1,000µl	10ml	750,000	4,500,000	150	45µl

<sup>1</sup>Suggested seeding density for cells = 14,000–80,000 cells per cm<sup>2</sup>.

**Table 2. Cell Growth Area for Various Cell Culture Vessels.**

Plate Size	Growth Area (cm <sup>2</sup> ) <sup>1</sup>	Relative Area <sup>2</sup>
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

<sup>1</sup>This information was calculated for Corning® culture dishes.


<sup>2</sup>Relative area is expressed as a factor of the growth area of the 96-well plate recommended for optimization studies. To determine the proper cell plating density, multiply 5,000–30,000 cells by this factor.




#### 4.B. Preparing the FuGENE® SI Transfection Reagent-RNA Complex

We strongly recommend that you optimize transfection conditions for each cell line; see Section 4.E. A ratio of 0.3µl of FuGENE® SI Transfection Reagent to 1pmol of siRNA works well for HEK293 cells in each well of a 96 well plate. For other cell types, the optimal amounts of FuGENE® SI Transfection Reagent and siRNA may differ. If you have optimized transfection parameters, use the empirically determined transfection conditions and adjust the FuGENE® SI Transfection Reagent volume and siRNA amount in this protocol accordingly. If you choose not to optimize transfection parameters, use the general conditions recommended below.

This protocol prepares 250µl of FuGENE® SI Transfection Reagent-RNA complex, which is sufficient to transfect cells in 25 wells of a 96-well plate, five wells of a 24-well plate, one 35mm culture dish or one well of a six-well plate when using 0.3µl of FuGENE® SI Transfection Reagent, 1pmol of siRNA and 10µl of transfection complex per well. To prepare sufficient volume for additional wells or larger culture vessels, scale up all volumes accordingly. See Tables 1 and 2.

 The FuGENE® SI Reagent-RNA complex must be prepared in serum-free medium even if cells are transfected in the presence of serum.

1. Before use, equilibrate FuGENE® SI Transfection Reagent and serum-free medium to room temperature.
2. Mix the FuGENE® SI Transfection Reagent by inverting or vortexing briefly.
3. Add 117.5µl of serum-free medium to a sterile tube or well of a U- or V-bottom plate. Add 7.5µl of FuGENE® SI Transfection Reagent directly to the medium. Immediately vortex at high speed for 1 second to mix.

 Add FuGENE® SI Transfection Reagent directly to the serum-free medium. Do not allow undiluted FuGENE® SI Transfection Reagent to contact the sides of the tube or plate.

**Note:** To avoid pipetting small volumes of reagent, you can perform serial dilution of the reagent in serum-free medium. Use intermediate dilutions immediately and discard any unused intermediate dilutions.

4. Add 122.5µl of serum-free medium to a sterile tube or well of a U- or V-bottom plate. Add 2.5µl of 10µM RNA, and vortex briefly to mix.

**Note:** The concentration of diluted RNA is 200nM. This results in an approximately 10nM siRNA during transfection.

5. Combine 125µl of diluted FuGENE® SI Transfection Reagent prepared in Step 3 and 125µl of diluted siRNA prepared in Step 4 in a sterile tube or U- or V-bottom plate. Immediately vortex to mix.
6. Incubate the FuGENE® SI Transfection Reagent-RNA complex for 5–15 minutes at room temperature. Proceed to Section 4.C to transfect cells using the traditional or rapid protocol. Proceed to Section 4.D to transfect cells using the reverse protocol.

**Note:** The optimal incubation time is typically 5–15 minutes, depending on the cell line used. We do not recommend incubation times longer than 15 minutes.

#### 4.C. Traditional or Rapid Transfection Protocol

We strongly recommend that you optimize transfection conditions for each cell line. If you have optimized transfection conditions as described in Section 4.E, use the empirically determined conditions and adjust this protocol accordingly. If you choose not to optimize transfection conditions, use the general conditions recommended below.

1. Add 10 $\mu$ l of the FuGENE<sup>®</sup> SI Transfection Reagent-RNA complex prepared in Section 4.B per well of a 96-well plate containing 100 $\mu$ l of cells in growth medium. Add the transfection complex to cells in a dropwise manner. Ensure complete mixing and even distribution over the entire surface by pipetting, using a plate shaker or rocking flasks.

**Note:** The growth medium volume may vary, depending on well format and standard laboratory practices.

2. Incubate cells for 24–72 hours.

**Note:** The optimal incubation time depends on the gene target, cell line being transfected, and downstream assay used to evaluate the effects of the RNA.

3. Measure the siRNA effect using your chosen analysis method.

#### 4.D. Reverse Transfection Protocol

We strongly recommend that you optimize transfection conditions for each cell line. If you have optimized transfection conditions as described in Section 4.E, use the empirically determined conditions and adjust this protocol accordingly. If you choose not to optimize transfection conditions, use the general conditions recommended below.

1. Add 10 $\mu$ l of FuGENE<sup>®</sup> SI Transfection Reagent-RNA complex prepared in Section 4.B to each well of a 96-well plate.
2. Add 100 $\mu$ l of cells prepared in Section 4.A. Ensure complete mixing and even distribution by pipetting, using a plate shaker or rocking flasks.

**Note:** The cell volume in growth medium may vary, depending on well format and standard laboratory practice.

3. Incubate cells for 24–72 hours.

**Note:** The optimal incubation time depends on the gene target, cell line being transfected, and downstream assay used to evaluate the effects of the RNA.

4. Measure the siRNA effect using your chosen analysis method.


#### 4.E. Transfection Optimization

The optimal transfection conditions are those that result in the lowest toxicity and largest RNA effect. This protocol allows you to optimize transfection conditions using the desired siRNA sequence and your chosen method to quantify the effect of RNA on cells.

For the initial optimization, we recommend testing 0.1–10.0pmol of siRNA and 0.3–0.6µl of FuGENE® SI Transfection Reagent per well of a 96-well plate using standard growth conditions for your cell type. See Figure 2 for an example plate layout. If 0.6µl of FuGENE® SI Transfection Reagent volume yields the highest transfection efficiency, we recommend performing a second round of optimization with reagent volumes higher than 0.6µl. Likewise, if a volume of 0.3µl yields the highest transfection efficiency, we recommend testing lower volumes of reagent.

Include the following controls when optimizing transfection conditions:

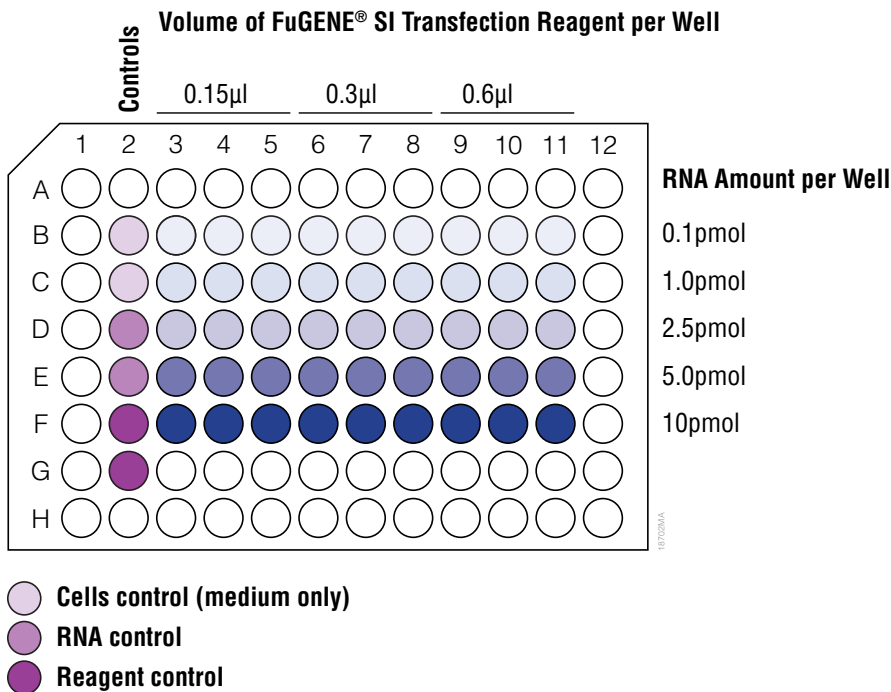
<b>Cells control</b>	Medium alone (e.g., 10µl of serum-free medium)
<b>RNA control</b>	RNA without FuGENE® SI Transfection Reagent (e.g., 1pmol of RNA in a 10µl volume)
<b>Reagent control</b>	FuGENE® SI Transfection Reagent without RNA (e.g., 0.3µl of reagent in 10µl of serum-free medium)

- Calculate the total amount of cells, siRNA, FuGENE® SI Transfection Reagent and each FuGENE® SI Transfection Reagent-RNA complex required for optimization. Include any controls when performing these calculations.  
**Note:** Prepare one tube of FuGENE® SI Transfection Reagent-RNA complex for each combination of RNA amount and reagent volume.
- Prepare 100µl of cells in growth medium per well as described in Section 4.A.  
**Note:** The optimal number of cells can vary, depending on the cell line, transfection protocol used, cell culture conditions and other factors. See Section 3.F for more information.
- In a sterile polystyrene tube or a U- or V-bottom plate, prepare each FuGENE® SI Transfection Reagent-RNA complex using serum-free medium as described in Section 4.B. Adjust the volume of serum-free medium used to dilute the FuGENE® SI Transfection Reagent to account for differences in the reagent volume.  
 Add FuGENE® SI Transfection Reagent directly to medium; do not allow undiluted FuGENE® SI Transfection Reagent to contact the sides of the tube or plate.  
**Note:** We suggest starting with a 5-minute incubation when forming the FuGENE® SI Transfection Reagent-RNA complex. However, the optimal incubation time may be 5–15 minutes, depending on the cell line used. We do not recommend incubation times longer than 15 minutes.
- Briefly vortex or mix the FuGENE® SI Transfection Reagent-RNA complexes. Add 10µl of the appropriate transfection complex to cells in the 96-well plate. Add the transfection complex to cells in a drop-wise manner. Ensure complete mixing and even distribution over the entire surface by pipetting, using a plate shaker or rocking flasks.  
**Note:** Other volumes may be optimal, depending on the transfection conditions. We recommend transfecting cells with 2–10µl of transfection complex per well of a 96-well plate to determine the optimal volume.

5. Return cells to the incubator for 24–72 hours.

**Note:** The optimal incubation time depends on the gene target, cell line being transfected and downstream assay used to evaluate the effect of the RNA.

6. Use your chosen analysis method to identify the conditions that have the largest RNA effect.



**Figure 2. Plate layout for transfection optimization.**

## 5. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: [www.promega.com](http://www.promega.com). E-mail: [techserv@promega.com](mailto:techserv@promega.com)

### Symptoms

Little or no RNA effect

### Causes and Comments

Poor-quality RNA or insufficient RNA quantity was used.

- Verify the amount, purity and sequence of RNA.
- Ensure that the RNA preparation does not contain chemical contaminants at levels that adversely affect transfection efficiency and cell growth. If necessary, clean up the RNA to remove any contaminants that carried over from RNA synthesis including salts, ethanol and proteins.

FuGENE® SI Transfection Reagent was dispensed into aliquots. Check that the FuGENE® SI Transfection Reagent is stored in the original glass vials. If dispensed into plastic containers, the reagent can lose activity.

FuGENE® SI Transfection Reagent came into contact with plastic or was inadequately mixed when reagent was diluted in transfection medium in Section 4.B. Repeat transfection, carefully pipetting undiluted FuGENE® SI Transfection Reagent directly into transfection medium and avoiding the sides of the container. Mix vigorously. If the FuGENE® SI Transfection Reagent is mixed too gently, the reagent may form a layer on top of the medium and make contact with the plastic.

Transfection efficiency was low due to suboptimal transfection conditions, including FuGENE® SI Transfection Reagent volume, RNA amount, transfection complex incubation time, volume of transfection complex. Optimize these transfection conditions as described in Section 4.E. For optimal results, other transfection conditions may require optimization; see Section 3.

Transfection efficiency was low because transfection complex was formed in serum-containing medium. Use transfection medium that does not contain serum when forming the FuGENE® SI Transfection Reagent-RNA complex and repeat the experiment. Alternatively, form the transfection complex in sterile water.

**Symptoms**

Little or no RNA effect (continued)

**Causes and Comments**

Transfection efficiency was low because the cell number was too high or cells were in post-log phase. When confluent cultures are subcultured or cells are plated at a density that is too high, cells fail to divide in the cell culture being transfected. Only use log-phase cell cultures to prepare cells prior to transfection, and seed cultures at the proper density for transfection.

Transfection efficiency was low due to an insufficient number of cells. Use cells that are at least 25% confluent; increase cell density if necessary. Low cell density results in fewer cells available to take up transfection complex, and excess complex may be cytotoxic.

Transfection efficiency was low because the medium or a medium component interfered with transfection.

- Different media and medium components can affect transfection efficiency and subsequent growth of transfected cells. We do not recommend using additives such as antimicrobial agents in the medium unless you have previously tested them in the cell type being transfected.
- Lot-to-lot differences in the quality of serum and medium can affect transfection efficiency. Check that the medium and/or serum is from the same lot that worked previously. Try new lots or a different vendor.

Cell culture was contaminated with mycoplasma. Cultures contaminated with mycoplasma have decreased transfection efficiency. Determine if culture is contaminated with mycoplasma using a commercially available mycoplasma contamination assay.

Inconsistent results

Transfection efficiency was inconsistent because transfection conditions were at the edge of a performance plateau. Optimize the volume of reagent, RNA amount, volume of transfection complex, incubation time for complex formation and other transfection conditions as described in Section 4.E. To increase consistency, use transfection conditions in the middle of any performance plateaus. If conditions are at a plateau edge, very small procedural differences can cause large differences in transfection efficiency.

**5. Troubleshooting (continued)**

**Symptoms**

**Causes and Comments**

Inconsistent results (continued)

Cells were not properly cultured or maintained. Cells can change with culture conditions such as passage level, passage conditions, medium used and serum used. For some cell lines, these changes have little to no effect on transfection efficiency, but for other cell lines, they have profound effects. If necessary, evaluate the effect that cell culture conditions have on transfection of your particular cell type, and optimize cell culture conditions.

Signs of cytotoxicity

RNA resulted in cell cytotoxicity due to effects related to the RNA sequence (e.g., RNA interference). To evaluate cytotoxicity and detect signs of reduced viability or poor cell health, perform the following controls:

- Cells that are not transfected
- Cells treated with RNA alone (e.g., without FuGENE® SI Transfection Reagent)

Compare viability and/or morphology of transfected cells and control cells. If transfected cells show signs of cytotoxicity but control cells do not, optimize transfection conditions including RNA amount, volume of transfection complex added to cells, cell number and transfection complex incubation time to identify conditions that result in less cytotoxicity. If cytotoxicity levels are still unacceptable, consider choosing a different RNA sequence or gene target.

The FuGENE® SI Transfection Reagent resulted in cell cytotoxicity. To detect signs of cytotoxicity or poor cell health, perform the following controls:

- Cells that are not transfected
- Cells treated with FuGENE® SI Transfection Reagent alone (e.g., with no RNA added)

Compare viability and/or morphology of transfected cells and control cells. If transfected cells show signs of cytotoxicity but control cells do not, optimize transfection conditions, including volume of FuGENE® SI Transfection Reagent, volume of transfection complex added to cells, cell number and transfection complex incubation time to identify conditions that result in less cytotoxicity and acceptable transfection efficiency.

**Symptoms**

Signs of cytotoxicity (continued)

**Causes and Comments**

Volume of transfection complex was too high for the number of cells. Increase the number of cells plated, decrease the volume of transfection complex added to cells or both. Evaluate different volumes of reagent per transfection and transfection complex incubation times. Evaluate different volumes of transfection complex added to the cells; for example, prepare the transfection complex as usual, but add 2µl, 5µl and 8µl of transfection complex to each well. See Section 4.E for more information about optimizing transfection conditions.

Cell culture was contaminated with mycoplasma. Cell cultures contaminated with mycoplasma have decreased transfection efficacy. Determine if cell culture is contaminated with mycoplasma using a commercially available mycoplasma contamination assay.

Cells were not healthy due to cell culture conditions. Assess the physiological state of cells and incubation conditions (e.g., check the incubator CO<sub>2</sub>, humidity and temperature levels). Observe cells prior to each passage to check cellular morphology and confirm an absence of contaminants. Make sure cells are not overgrown. Routinely passage cells prior to reaching confluency. Make sure that growth medium and any additives are not expired and were stored properly.

Transfection medium was toxic to cells. For example, DMEM is toxic to some insect cell lines. Use serum-free growth medium that is appropriate for your cell line as the transfection medium. Alternatively, prepare the transfection complex in sterile water.

**6. Related Products**
**Detection Instruments**

<b>Product</b>	<b>Cat.#</b>
GloMax <sup>®</sup> Discover System	GM3000
GloMax <sup>®</sup> Explorer System	GM3500
GloMax <sup>®</sup> Navigator System	GM2000
Quantus <sup>™</sup> Fluorometer	E6150



## 6. Related Products (continued)

### RNA Quantitation

Product	Size	Cat.#
QuantiFluor® RNA System	1ml	E3310

### RT-qPCR Systems

Product	Size	Cat.#
GoTaq® 1-Step RT-qPCR System	5ml	A6020
GoTaq® 2-Step RT-qPCR System	5ml	A6010
GoTaq® Probe 1-Step RT-qPCR System	2ml	A6120
GoTaq® Probe 2-Step RT-qPCR System	2ml	A6110

Additional sizes available.

### RNAi Analysis

Product	Size	Cat.#
T7 RiboMAX™ Express RNAi System		P1700
siCHECK™-2 Vector		C8021
pmirGLO Dual-Luciferase miRNA Target Expression Vector		E1330

### 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

For Laboratory Use. Additional sizes available.

## Luciferase Assay Systems

<b>Product</b>	<b>Size</b>	<b>Cat.#</b>
Steady-Glo <sup>®</sup> Luciferase Assay System	10ml*	E2510
Bright-Glo <sup>™</sup> Luciferase Assay System**	10ml*	E2610
ONE-Glo <sup>™</sup> Luciferase Assay System**	10ml*	E6110
Dual-Luciferase <sup>®</sup> Reporter Assay System	100 assays*	E1910
Luciferase Assay System	100 assays*	E1500
Luciferase Assay Reagent	1,000 assays	E1483
<i>Renilla</i> Luciferase Assay System	100 assays*	E2810
QuantiLum <sup>®</sup> Recombinant Luciferase	1mg*	E1701
EnduRen <sup>™</sup> Live Cell Substrate	0.34mg*	E6481
ViviRen <sup>™</sup> Live Cell Substrate	0.37mg*	E6491

\*Available in additional sizes.

\*\*For Laboratory Use.

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