# SuperFect® Transfection Reagent Handbook

For high transfection efficiencies in a broad range of cell lines

December 2002

QIAGEN

## QIAGEN Worldwide

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## QIAGEN Distributors

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# Contents

## Kit Contents

1.2 ml SuperFect® Transfection Reagent (3 mg/ml), sufficient for 40 transfections in 60 mm dishes or 160 transfections in 12-well plates, following the standard protocol

**or**

4 x 1.2 ml SuperFect Transfection Reagent (3 mg/ml), sufficient for 160 transfections in 60 mm dishes or 640 transfections in 12-well plates, following the standard protocol.

## Shipping and Storage

SuperFect Transfection Reagent is supplied as a ready-to-use solution and shipped at ambient temperature, without loss in stability. However, it should be stored at 2–8°C upon arrival. SuperFect Transfection Reagent is stable for 1 year at 2–8°C.

## Product Use Limitations

SuperFect Transfection Reagent is developed, designed and sold for research purposes only. It is not to be used for human diagnostic or drug purposesor to be administered to human unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this text.

## Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department. We will credit your account or exchange the product — as you wish.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see inside front cover).

# Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN® products. If you have any questions or experience any difficulties regarding SuperFect Transfection Reagent, or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are also a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see inside front cover).

# Quality Control

Endotoxin levels are <10 EU/ml determined using a Kinetic-QCL test (BioWhittaker, Inc). SuperFect Transfection Reagent is tested by transfection of pCMVβ into HeLaS3 and COS-7 cells to ensure lot-to-lot consistency. Microbial limit tests guarantee absence of any contaminating bacteria or fungi. Homogeneity of SuperFect Reagent is tested by gel permeation chromatography.

# Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/ts/msds.asp**, where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

**24-hour emergency information**

Emergency medical information in English, French, and German can be obtained 24 hours a day from: Poison Information Center Mainz, Germany

Tel: +49-6131-19240

# Introduction

SuperFect Transfection Reagent represents a new class of activated-dendrimer transfection reagent designed for outstanding transfection results. SuperFect Transfection Reagent offers significant advantages over many other transfection methods.

**SuperFect Transfection Reagent offers:**

- Fast procedure — transfection-complex formation in just 5–10 minutes
- Transfection in a broad range of cell lines
- High transfection efficiencies through novel molecular design
- Excellent reproducibility
- Decreased cytotoxicity

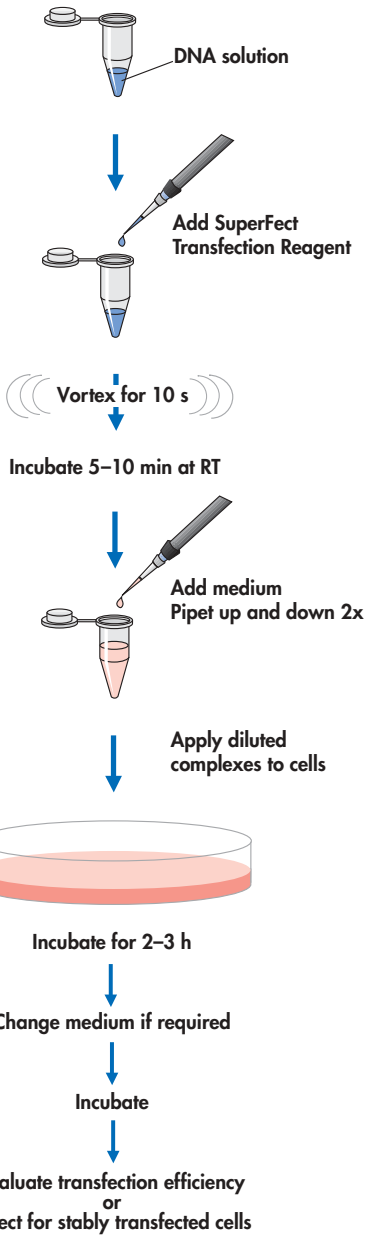
**Principle and procedure**

SuperFect Transfection Reagent is a specifically designed activated dendrimer (1). SuperFect Reagent possesses a defined spherical architecture, with branches radiating from a central core and terminating at charged amino groups. SuperFect Reagent assembles DNA into compact structures, optimizing the entry of DNA into the cell. SuperFect–DNA complexes possess a net positive charge, which allows them to bind to negatively charged receptors (e.g., sialylated glycoproteins) on the surface of eukaryotic cells. Once inside, SuperFect Reagent buffers the lysosome after it has fused with the endosome, leading to pH inhibition of lysosomal nucleases and stability of SuperFect–DNA complexes.

**Broad cell line spectrum**

SuperFect Transfection Reagent has been used for transfection of a variety of different cell lines, and yields significantly higher transfection efficiencies than many other transfection methods. A searchable list of cell lines successfully transfected using SuperFect Reagent, as well as customer-developed protocols for transfection of various cell lines using SuperFect Reagent, is available at the Transfection Tools web site — **www.qiagen.com/transfectiontools/**

## Transfection Procedure for SuperFect Transfection Reagent



# Important Notes

Transfection efficiencies are controlled by a variety of different parameters. The following factors should be considered carefully.

### Cell culture

A healthy cell culture lays the foundation for successful transfection. Different cells or cell lines have very specific media, serum, and supplement requirements. Low passage number (<50 splitting cycles) ensures that the cell genotype has not become altered. Highest transfection efficiencies are obtained using the confluence levels indicated in the appropriate protocol sections. We recommend subculturing cells 24 hours before transfection. This provides normal cell metabolism and increases the likelihood of DNA uptake. Contamination with bacteria, for example mycoplasma, and fungi should be avoided, since it can drastically alter transfection results. Antibiotics can be included in the medium used for transfection with SuperFect Reagent, and during subsequent incubation for gene expression.

### Effect of serum

In contrast to many liposomal transfection reagents, the presence of serum during transfection with SuperFect Reagent significantly increases transfection efficiencies. Therefore, we recommend using the percentage of serum that cells have been adapted to in culture during the incubation of cells with transfection complexes, and during the subsequent incubation for gene expression. However, we do not recommend using serum during complex formation between SuperFect Reagent and plasmid DNA, as serum may inhibit complex formation.

### Vector construct

The type of transfection vector (plasmid DNA, PCR products, oligonucleotides) influences the transfection results. The configuration and size of the construct also determine the efficiency of transfection. Transient transfection is most efficient with supercoiled plasmid DNA. In stable transfection, linear DNA yields optimal integration of the DNA into the host genome, but results in lower DNA uptake by the cells, relative to supercoiled DNA.

### Plasmid DNA quality

Plasmid DNA quality strongly influences several transfection parameters, such as efficiency, reproducibility, and toxicity, as well as interpretation of results. Therefore only plasmid DNA of the highest purity should be used. DNA purified using HiSpeed®, QIAfilter™, and QIAGEN Plasmid Kits is well suited for transfection of most cell lines. For highest reproducibility and best results with all cell lines, we recommend DNA purified using the EndoFree® Plasmid Kit. This kit quickly and efficiently removes bacterial endotoxins during the plasmid purification procedure, ensuring optimal transfection results.

# Transfection Optimization

The protocols provided on pages 13–20 yield high transfection levels for many tested cell lines. However, to achieve optimal transfection efficiency for every new cell line/plasmid DNA combination used, it is recommended to optimize a number of parameters. These are: the amounts of SuperFect Reagent, DNA, and SuperFect–DNA complex; the cell number/confluency prior to transfection; and the length of exposure of cells to SuperFect–DNA complexes. Once the parameters yielding maximum transfection efficiency have been determined, they should be kept constant in every experiment using a particular cell line/plasmid DNA combination.

### Cell density at the time of complex addition

**For adherent cells**, the optimal confluency at the time of transfection-complex addition is normally 40–80%. The optimal confluency should be determined for every new cell line to be transfected, and kept constant in future experiments. This is achieved by counting cells prior to seeding and by keeping the time period between seeding and transfection constant. Table 1 lists the recommended number of adherent cells to seed per culture plate/dish **the day before transfection**.

**For suspension cells**, split the cells the day before the transfection experiment. This will ensure that the cell density is not too high and that the cells are in optimal physiological condition on the day of transfection. Table 1 lists the recommended number of suspension cells to seed per culture plate/dish and the volume of medium to use **the day of transfection**.

**Table 1. Recommended number of cells per culture vessel for transfection. Volumes given apply to each well of multiwell plates.**

| Culture format | Adherent cells to seed*† (day before transfection) | Suspension cells to seed* (day of transfection) | Volume of medium (µl) |
|---|---|---|---|
| 96-well plate | $0.5–2.0 \times 10^4$ | $0.5–2.0 \times 10^5$ | 100 |
| 48-well plate | $1.0–4.0 \times 10^4$ | $1.0–3.5 \times 10^5$ | 150 |
| 24-well plate | $2.0–8.0 \times 10^4$ | $2.0–7.0 \times 10^5$ | 350 |
| 12-well plate | $0.4–2.0 \times 10^5$ | $0.5–1.5 \times 10^6$ | 800 |
| 6-well plate | $0.9–4.0 \times 10^5$ | $1.0–3.5 \times 10^6$ | 1600 |
| 60 mm dish | $2.0–8.0 \times 10^5$ | $2.5–7.5 \times 10^6$ | 4000 |
| 100 mm dish | $0.5–2.5 \times 10^6$ | $0.5–2.0 \times 10^7$ | 7000 |

* *Actual values depend on cell type and size.*

† *The volume of medium used to seed adherent cells the day before transfection is not critical. This medium is aspirated on the day of transfection and replaced by the diluted transfection complexes. When seeding adherent cells, use a volume of medium suitable for your cell culture format.*

### Amount of DNA

The optimal quantity of plasmid DNA for transfection is determined by the properties of the transfected plasmid, which include the type of promoter, origin of replication, and plasmid size. Toxic effects may arise if too much plasmid with a high expression rate is used. Conversely, if insufficient plasmid with a low expression rate is used, transfection efficiency may be too low. Therefore, optimization of plasmid DNA concentration should be performed for every new plasmid and/or new cell line used. The recommended amount of DNA for transfection with SuperFect Reagent in 60 mm dishes is 2.5–10 µg.

A pipetting scheme for optimizing transfection of adherent and suspension cells in 60 mm dishes is provided in Table 2. For transfection using other culture formats, please refer to Tables 3 and 4 on pages 13 and 17, respectively.

### Ratio of SuperFect Reagent to DNA

The overall charge of the SuperFect–DNA complex is determined by the ratio of SuperFect Reagent to DNA. Optimal binding of SuperFect–DNA complexes to the negatively charged groups (e.g. sialylated glycoproteins) on the cell surface requires a slightly positive net charge. The ratio of SuperFect Reagent (µl) to DNA (µg) is an important factor to optimize for every new cell line and DNA construct used.

**Note:** SuperFect–DNA complex formation should be performed in the absence of serum, antibiotics, and proteins.

A pipetting scheme for optimizing transfection of adherent and suspension cells in 60 mm dishes is provided in Table 2. For transfection using other culture formats, please refer to Tables 3 and 4 on pages 14 and 18, respectively.

**Table 2. Pipetting scheme for optimizing transfection of adherent and suspension cells in 60 mm dishes**

| DNA (µg) | Ratio of DNA to SuperFect Reagent (SF) 1:2 | 1:5 | 1:10 |
|---|---|---|---|
| 2.5 | 2.5 µg DNA<br>5 µl SF | 2.5 µg DNA<br>12.5 µl SF | 2.5 µg DNA<br>25 µl SF |
| 5.0 | 5 µg DNA<br>10 µl SF | 5 µg DNA<br>25 µl SF | 5 µg DNA<br>50 µl SF |
| 10.0 | 10 µg DNA<br>20 µl SF | 10 µg DNA<br>50 µl SF | 10 µg DNA<br>100 µl SF |

### Incubation period with SuperFect–DNA complexes

**For adherent cells**, the length of incubation of transfection complexes with cells should be optimized by varying the incubation time within a range of 1–16 hours. Optimal results are typically obtained with incubation times of 2–3 hours.

**For suspension cells**, experiments have shown that in most cases, removal of SuperFect–DNA transfection complexes is not necessary. However, if cytotoxicity is observed, remove the transfection complexes by centrifugation after a 2–3 hour incubation period. Remove the medium from the cell pellet, resuspend cells in fresh medium (containing serum and antibiotics), and incubate for gene expression.

### Transfection of primary cells

SuperFect Reagent can be used for transfection of primary cells with some modifications to the standard protocols. The amounts of DNA and SuperFect Reagent to use for transfection of primary cells are given in appropriate positions in the protocols.

### Transfection in multiwell plates — preparing a master mix

If you are performing transfection in multiwell plates, prepare a transfection complex master mix for distribution into plate wells.

- Calculate the required volumes of each component and the total volume before you prepare the master mix.
- Prepare 10% more master mix than is required to allow for pipetting errors, i.e., for a 48-well plate prepare enough master mix for 53 wells.
- Add and mix the components of the master mix according to the instructions in the protocol.
- Use a repeat pipet to distribute transfection complexes.

# Protocol: Transient Transfection of Adherent Cells

The following protocol is for transient transfection of adherent cells in 60 mm dishes. Starting points for optimizing transient transfection in other formats are listed in Table 3 on page 14. See Table 1 on page 10 for the recommended number of cells to seed in other culture formats. Optimal transfection conditions should be determined for every cell line if the highest transfection efficiency with SuperFect Reagent is required. Please refer to the optimization guidelines on pages 10–12.

1. **The day before transfection, seed 2–8 x $10^5$ cells (depending on the cell type) per 60 mm dish in 5 ml appropriate growth medium. The cell number seeded should produce 40–80% confluence on the day of transfection.**
2. **Incubate the cells under their normal growth conditions (generally 37°C and 5% $CO_2$).**
3. **On the day of transfection, dilute 5 µg DNA dissolved in TE buffer pH 7 to pH 8 (minimum DNA concentration: 0.1 µg/µl) with cell growth medium containing no serum, proteins, or antibiotics to a total volume of 150 µl. (For primary cells, use 2.5 µg plasmid DNA). Mix and spin down the solution for a few seconds to remove drops from the top of the tube.**

   **IMPORTANT:** Serum and antibiotics present during this step will interfere with complex formation and will significantly decrease transfection efficiency.

   **Note:** Plasmid DNA quality strongly influences several transfection parameters such as efficiency, reproducibility, and toxicity, as well as interpretation of results. Therefore, only plasmid DNA of the highest purity should be used. DNA purified using HiSpeed, QIAfilter, and QIAGEN Plasmid Kits is well suited for transfection of most cell lines. For highest reproducibility and best results with all cell lines, we recommend DNA purified using the EndoFree Plasmid Kit. This kit quickly and efficiently removes bacterial endotoxins during the plasmid purification procedure, ensuring optimal transfection results.
4. **Add 30 µl SuperFect Transfection Reagent to the DNA solution. (For primary cells, use 15 µl SuperFect Reagent). Mix by pipetting up and down 5 times, or by vortexing for 10 s.**

   **Note:** It is not necessary to keep SuperFect Reagent on ice at all times. 10–15 min at room temperature will not alter its stability.
5. **Incubate the samples for 5–10 min at room temperature (15–25°C) to allow transfection-complex formation.**
6. **While complex formation takes place, gently aspirate the growth medium from the dish, and wash cells once with 4 ml PBS.**

7. **Add 1 ml cell growth medium (containing serum and antibiotics) to the reaction tube containing the transfection complexes. Mix by pipetting up and down twice, and immediately transfer the total volume to the cells in the 60 mm dishes.**

   At this stage serum and antibiotics no longer interfere with, but significantly enhance the transfection efficiency of SuperFect Reagent.

8. **Incubate cells with the transfection complexes for 2–3 h under their normal growth conditions.**

9. **Remove medium containing the remaining complexes from the cells by gentle aspiration, and wash cells once with 4 ml PBS.**

10. **Add fresh cell growth medium (containing serum and antibiotics). Assay cells for expression of the transfected gene after an appropriate incubation time.**

    For example, cells transfected with *β-gal* or *cat* reporter constructs are typically incubated for 24–48 h after transfection to obtain maximal levels of gene expression.

**Table 3. Starting points for optimizing transient transfection of adherent cells in different culture formats. Volumes given apply to each well of multiwell plates.**

| Culture format | DNA (µg) | Final volume of DNA diluted in serum-free medium (µl) | Volume of SuperFect Reagent (µl) | Volume of serum-containing medium (µl)* |
|---|---|---|---|---|
| **Protocol step** | **3** | **3** | **4** | **7** |
| 96-well plate | 0.50 | 30 | 2.5† | 150 |
| 48-well plate | 0.75 | 50 | 4.5† | 250 |
| 24-well plate | 1.0 | 60 | 5.0 | 350 |
| 12-well plate | 1.5 | 75 | 7.5 | 400 |
| 6-well plate | 2.0 | 100 | 10.0 | 600 |
| 60 mm dish | 5.0 | 150 | 30.0 | 1000 |
| 100 mm dish | 10.0 | 300 | 60.0 | 3000 |

* *Medium should contain the same percentage of serum as routinely used for culturing cells.*

† *If transfections are performed in 96- or 48-well plates, dilute SuperFect Reagent with cell growth medium containing no serum to a total volume of 20 µl or 50 µl, respectively, before addition to the diluted DNA solution prepared in step 3.*

# Protocol: Stable Transfection of Adherent Cells

The following protocol is for stable transfection of adherent cells in 60 mm dishes. See Table 1 on page 10 for the recommended number of cells to seed in other culture formats. Optimal transfection conditions should be determined for every cell line if the highest transfection efficiency with SuperFect Reagent is required. Please refer to the optimization guidelines on pages 10–12.



1. **The day before transfection, seed 2–8 x $10^5$ cells (depending on the cell type) in 5 ml appropriate growth medium in 60 mm dishes. The cell number seeded should produce 40–80% confluence on the day of transfection.**
2. **Incubate the cells under their normal growth conditions (generally 37°C and 5% $CO_2$).**
3. **On the day of transfection, dilute 5 µg DNA dissolved in TE buffer pH 7 to pH 8 (minimum DNA concentration: 0.1 µg/µl) with cell growth medium containing no serum, proteins, or antibiotics to a total volume of 150 µl. (For primary cells, use 2.5 µg plasmid DNA). Mix and spin down the solution for a few seconds to remove drops from the top of the tube.**

   **IMPORTANT:** Serum and antibiotics present during this step will interfere with complex formation and will significantly decrease transfection efficiency.

   **Note:** Plasmid DNA quality strongly influences several transfection parameters such as efficiency, reproducibility, and toxicity, as well as interpretation of results. Therefore, only plasmid DNA of the highest purity should be used. DNA purified using HiSpeed, QIAfilter, and QIAGEN Plasmid Kits is well suited for transfection of most cell lines. For highest reproducibility and best results with all cell lines, we recommend DNA purified using the EndoFree Plasmid Kit. This kit quickly and efficiently removes bacterial endotoxins during the plasmid purification procedure, ensuring optimal transfection results.
4. **Add 20 µl SuperFect Transfection Reagent to the DNA solution. (For primary cells, use 15 µl SuperFect Reagent). Mix by pipetting up and down 5 times, or by vortexing for 10 s.**

   **Note:** It is not necessary to keep SuperFect Reagent on ice at all times. 10–15 min at room temperature will not alter its stability.
5. **Incubate the samples for 5–10 min at room temperature (15–25°C) to allow transfection-complex formation.**
6. **While complex formation takes place, gently aspirate the growth medium from the dish, and wash cells once with 4 ml PBS.**
7. **Add 1 ml cell growth medium (containing serum and antibiotics) to the reaction tube containing the transfection complexes. Mix by pipetting up and down twice, and immediately transfer the total volume to the cells in the 60 mm dishes.**

   At this stage serum and antibiotics no longer interfere with, but significantly enhance the transfection efficiency of SuperFect Reagent.

8. **Incubate cells with the transfection complexes for 2–3 h under their normal growth conditions.**
9. **Remove medium containing the remaining complexes from the cells by gentle aspiration, and wash cells 3–4 times with 4 ml PBS.**
10. **Add fresh cell growth medium (containing serum and antibiotics), and incubate for 24–48 h.**
11. **Passage cells at 1:10 to 1:15 into the appropriate selective medium. Maintain cells in selective medium under their normal growth conditions until colonies appear.**

    **Note:** We recommend establishing a kill curve (dose-response curve) with each combination of cell line and antibiotic used. It is important to bear in mind that the kill curve can be influenced by cell density.

    It may be necessary to plate the transfected cells into their normal medium (i.e., with no selective drug) and then incubate them for 1–2 days before addition of selective medium.

# Protocol: Transient Transfection of Suspension Cells

The following protocol is for transient transfection of suspension cells in 60 mm dishes. Starting points for optimizing transient transfection in other formats are listed in Table 4 on page 18. See Table 1 on page 10 for the recommended number of cells to seed and the volume of medium to use in other culture formats. Optimal transfection conditions should be determined for every cell line if the highest transfection efficiency with SuperFect Reagent is required. Please refer to the optimization guidelines on pages 10–12.

1. **Split the cells the day before transfection.**
2. **On the day of transfection, harvest cells by centrifugation, remove the medium, and wash the cells once with PBS in a 10 ml Falcon® tube.**
3. **Seed 2.5–7.5 x $10^6$ cells (depending on the cell type) per 60 mm dish in 4 ml cell growth medium containing serum and antibiotics.**
4. **Dilute 5 µg DNA dissolved in TE buffer pH 7 to pH 8 (minimum DNA concentration: 0.1 µg/µl) with cell growth medium containing no serum, proteins, or antibiotics to a total volume of 150 µl. Mix and spin down the solution for a few seconds to remove drops from the top of the tube.**

   **IMPORTANT:** Serum and antibiotics present during this step will interfere with complex formation and will significantly decrease transfection efficiency.

   **Note:** Plasmid DNA quality strongly influences several transfection parameters, such as efficiency, reproducibility, and toxicity, as well as interpretation of results. Therefore, only plasmid DNA of the highest purity should be used. DNA purified using HiSpeed, QIAfilter, and QIAGEN Plasmid Kits is well suited for transfection of most cell lines. For highest reproducibility and best results with all cell lines, we recommend DNA purified using the EndoFree Plasmid Kit. This kit quickly and efficiently removes bacterial endotoxins during the plasmid purification procedure, ensuring optimal transfection results.
5. **Add 20 µl SuperFect Reagent to the DNA solution. Mix by pipetting up and down 5 times, or by vortexing for 10 s.**

   **Note:** It is not necessary to keep SuperFect Reagent on ice at all times. 10–15 min at room temperature will not alter its stability.
6. **Incubate the samples for 5–10 min at room temperature (15–25°C) to allow transfection-complex formation.**
7. **Add 1 ml cell growth medium (containing serum and antibiotics) to the tube containing the transfection complexes. Mix by pipetting up and down twice and immediately add the transfection complexes drop-wise onto the cells in the 60 mm dishes. Gently swirl the dish to ensure uniform distribution of the complexes in the dish.**

   At this stage serum and antibiotics no longer interfere with, but significantly enhance the transfection efficiency of SuperFect Reagent, and in addition, serum is beneficial for cell survival.

8. **Incubate cells with the transfection complexes under their normal growth conditions (generally 37°C and 5% $CO_2$) for the appropriate time for expression of the transfected gene.**

   **Note:** Removal of transfection complexes is not usually necessary. However, if cytotoxicity is observed, remove the transfection complexes by centrifugation after a 2–3 h incubation period. Remove the medium from the cell pellet, resuspend cells in fresh medium containing serum and antibiotics, and continue incubation for an appropriate time for expression of the transfected gene.

   Cells transfected with *β-gal* or *cat* reporter constructs are typically incubated for 24–48 h after transfection to obtain maximal levels of gene expression.

9. **Harvest cells by centrifugation, and assay for gene expression.**

**Table 4. Starting points for optimizing transient transfection of suspension cells in different culture formats. Volumes given apply to each well of multiwell plates.**

| Culture format | DNA (µg) | Final volume of DNA diluted in serum-free medium (µl) | Volume of SuperFect Reagent (µl) | Volume of serum-containing medium (µl)* |
|---|---|---|---|---|
| **Protocol step** | **4** | **4** | **5** | **7** |
| 96-well plate | 0.50 | 30 | 2† | 0 |
| 48-well plate | 0.75 | 50 | 3† | 0 |
| 24-well plate | 1.0 | 60 | 4 | 100 |
| 12-well plate | 1.5 | 75 | 6 | 200 |
| 6-well plate | 2.0 | 100 | 8 | 400 |
| 60 mm dish | 5.0 | 150 | 20 | 1000 |
| 100 mm dish | 10.0 | 300 | 40 | 3000 |

* *Medium should contain the same percentage of serum as routinely used for culturing cells.*

† *If transfections are performed in 96- or 48-well plates, dilute SuperFect Reagent with cell growth medium containing no serum to a total volume of 20 µl or 50 µl, respectively, before addition of the diluted DNA solution prepared in step 3.*

# Protocol: Stable Transfection of Suspension Cells

The following protocol is for stable transfection of suspension cells in 60 mm dishes. See Table 1 on page 10 for the recommended number of cells to seed and the volume of medium to use in other culture formats. Optimal transfection conditions should be determined for every cell line if the highest transfection efficiency with SuperFect Reagent is required. Please refer to the optimization guidelines on pages 10-12.

1. **Split the cells the day before transfection.**
2. **On the day of transfection, harvest cells by centrifugation, remove the medium, and wash the cells once with PBS in a 10 ml Falcon tube.**
3. **Seed 2.5–7.5 x $10^6$ cells (depending on the cell type) per 60 mm dish in 4 ml cell growth medium containing serum and antibiotics.**
4. **Dilute 5 µg DNA dissolved in TE buffer pH 7 to pH 8 (minimum DNA concentration: 0.1 µg/µl) with cell growth medium containing no serum, proteins, or antibiotics to a total volume of 150 µl. Mix and spin down the solution for a few seconds to remove drops from the top of the tube.**

   **IMPORTANT:** Serum and antibiotics present during this step will interfere with complex formation and will significantly decrease transfection efficiency.

   **Note:** Plasmid DNA quality strongly influences several transfection parameters, such as efficiency, reproducibility, and toxicity, as well as interpretation of results. Therefore, only plasmid DNA of the highest purity should be used. DNA purified using HiSpeed, QIAfilter, and QIAGEN Plasmid Kits is well suited for transfection of most cell lines. For highest reproducibility and best results with all cell lines, we recommend DNA purified using the EndoFree Plasmid Kit. This kit quickly and efficiently removes bacterial endotoxins during the plasmid purification procedure, ensuring optimal transfection results.
5. **Add 20 µl SuperFect Reagent to the DNA solution. Mix by pipetting up and down 5 times, or by vortexing for 10 s.**

   **Note:** It is not necessary to keep SuperFect Reagent on ice at all times. 10–15 min at room temperature will not alter its stability.
6. **Incubate the samples for 5–10 min at room temperature (15–25°C) to allow transfection-complex formation.**
7. **Add 1 ml cell growth medium (containing serum and antibiotics) to the tube containing the transfection complexes. Mix by pipetting up and down twice, and immediately add the transfection complexes drop-wise onto the cells in the 60 mm dishes. Gently swirl the dish to ensure uniform distribution of the complexes in the dish.**

   At this stage serum and antibiotics no longer interfere with, but significantly enhance the transfection efficiency of SuperFect Reagent, and in addition, serum is beneficial for cell survival.

8. **Incubate cells with the transfection complexes under their normal growth conditions (generally 37°C and 5% $CO_2$) for 24–48 h .**

   **Note:** Removal of transfection complexes is not usually necessary. However, if cytotoxicity is observed, remove the transfection complexes by centrifugation after a 2–3 h incubation period. Remove the medium from the cell pellet, resuspend cells in fresh medium (containing serum and antibiotics), and incubate for an appropriate time (typically 24–48 h) for expression of the drug-resistance gene.

9. **Wash cells 3–4 times with 4 ml PBS. Passage cells into the appropriate selective medium to give a suitable cell density for selection in soft agar or for single-cell–cloning in 96-well plates (e.g., a 1:10 to 1:1000 split). Maintain cells in selective medium under their normal growth conditions until colonies appear.**

   **Note:** We recommend establishing a kill curve (dose-response curve) with each combination of cell line and antibiotic used. It is important to bear in mind that the kill curve can be influenced by cell density.

   It may be necessary to plate the transfected cells into their normal medium (i.e., with no selective drug) and then incubate them for 1–2 days before addition of selective medium.

# Troubleshooting Guide

The following troubleshooting guide is helpful if lower transfection efficiencies or higher cytotoxicity than expected is observed. Comments and suggestions are listed in the order in which they should be considered.

| | Comments and suggestions |
|---|---|
| **Low transfection efficiency** | |
| a) SuperFect Reagent to DNA ratio is suboptimal | If the ratio of SuperFect Reagent to DNA is suboptimal, the overall charge of the complexes may be negative, neutral or strongly positive, which can lead to inefficient adsorption to the cell surface. Optimize the SuperFect Reagent to DNA ratio according to the optimization section (page 10). |
| b) Insufficient amount of SuperFect–DNA complex | If the transfection efficiency is lower than expected and cytotoxicity acceptably low, increase the overall amount of SuperFect–DNA complexes. See pipetting scheme in Table 2 on page 11. |
| c) Incubation time for gene expression is suboptimal | Different cell types achieve maximal expression levels at different times post-transfection. This should be kept in mind when determining the length of incubation with the complexes. If the time point of maximal expression is not known for a particular cell line, a time course experiment may be necessary. |
| d) Vector influence | Factors such as the promoter, origin of replication, and plasmid size influence gene expression rate. The optimal quantity of plasmid DNA used for transfection is dependent on the expression rate of the plasmid. |
| e) Cell density at the time of SuperFect–DNA complex addition is too high | If cell density is too high during complex addition, cells may not be at the optimal phase of growth. This can lead to insufficient uptake of the complexes into the cells or insufficient expression of the gene of interest. For adherent cells, the optimal confluency at the time of transfection complex addition is normally 40–80% (page 10). |

| | | Comments and suggestions |
|---|---|---|
| f) | Poor DNA quality | Plasmid DNA used for transfection should be of high quality. Impurities present in the DNA preparation can potentially lower transfection efficiency. DNA should be purified using HiSpeed, QIAfilter, or QIAGEN Plasmid Kits or an equivalent method. For endotoxin-sensitive cell lines and primary cells, we recommend using DNA purified with EndoFree Plasmid Kits to ensure the highest transfection efficiencies. |
| g) | Reporter assay problem | Include positive controls to ensure that the reporter assay is working properly. |
| **Excessive cell death** | | |
| a) | Excessive exposure of cells to SuperFect–DNA complexes | Most adherent cell lines yield optimal results when incubated with SuperFect–DNA complexes for 2–3 hours. If sensitive adherent cells (e.g., primary cells) or cell lines demonstrate extensive cell death after treatment with SuperFect Reagent, reduce the exposure time of cells to complexes to 1 hour. For sensitive suspension cells or cell lines, remove the complexes via centrifugation after a 2–3-hour incubation, and wash cells carefully. With sensitive cell lines, we recommend 2–4 careful washing steps with complete medium rather than PBS. |
| b) | Amount of SuperFect–DNA complexes too high | If cell death continues after decreasing exposure times, decrease the amount of SuperFect–DNA complexes but keep the ratio of SuperFect Reagent to DNA constant (See pipetting scheme on page 11). |
| c) | Cells are stressed | In general, avoid stressing cells with temperature shifts and long periods without medium during washing steps. We recommend performing transfection experiments in the presence of serum, so that cells are not deprived of necessary growth factors and nutrients. |

| | | Comments and suggestions |
|---|---|---|
| d) | Vector related influences | Toxic effects may arise if a plasmid encoding a toxic protein is used, or if too much plasmid with a high expression rate is used. Conversely, if insufficient plasmid with a low expression rate is used, transfection efficiency may be too low. Optimization of plasmid DNA concentration, as described above and in the optimization section (page 10), should be performed for every new plasmid and/or new cell line used. |
| **Variable transfection efficiencies in replicate experiments** | | |
| a) | Inconsistent cell confluency in replicate experiments | Count cells prior to seeding to ensure that the same number of cells is seeded for each experiment. Keep incubation times between seeding and complex addition consistent between experiments. |
| b) | Possible mycopasma contamination | Mycoplasma contamination influences transfection efficiency. Variations in the growth behavior of mycoplasma-infected cells will lead to different transfection efficiencies between replicate experiments. |
| c) | Cells have been passaged too many times | Cells that have been passaged for an extended number of times tend to change their growth behavior, morphology and transfectability. When cells with high passage numbers are used for replicate experiments, decreased transfection efficiencies may be observed in later experiments. We recommend using cells with low passage number (< 50 splitting cycles). |
| d) | Serum variability | Variations in serum quality can lead to variation in transfection efficiency. In general, it is advisable to test a small lot of serum from a reputable supplier with a control cell line and assess it before performing transfection experiments. Once a given lot has yielded satisfactory and reproducible results, additional sera from the same lot should be purchased. |

# Appendix A: Composition of Buffers

| Buffer | Composition | Storage |
|---|---|---|
| 1x PBS (phosphate-buffered saline) | 137 mM NaCl<br>2.7 mM KCl<br>4.3 mM $Na_2HPO_4$<br>1.47 mM $KH_2PO_4$<br>Adjust to a final pH of 7.4 | room temp. |
| 1x TE buffer, pH 7.4 | 10 mM Tris·Cl, pH 7.4<br>1 mM EDTA | room temp. |

# Appendix B: Background Information

## Transfection principle

Transfection — delivery of foreign molecules such as DNA into eukaryotic cells — has become a powerful tool for the study and control of gene expression, e.g., for biochemical characterization, mutational analyses, or investigation of the effects of regulatory elements or cell growth behavior. Two principally different transfection techniques can be used; transient transfection and stable transfection. For further background information on transfection, please refer to current molecular biology manuals (2, 3).

### Transient transfection

When cells are transiently transfected, the DNA is introduced into the nucleus of the cell, but does not integrate into the chromosome. This means that many copies of the gene of interest are present, leading to high levels of expressed protein. Transcription of the transfected gene can be analyzed within 24 to 96 hours after introduction of the DNA depending on the construct used. Transient transfection is most efficient when supercoiled plasmid DNA is used.

### Stable transfection

With stable or permanent transfection, the transfected DNA is either integrated into the chromosomal DNA or maintained as an episome. Although linear DNA yields optimal integration of the DNA into the host genome, it lowers DNA uptake by the cells relative to supercoiled DNA. Cells which have successfully integrated the DNA of interest or have maintained episomal plasmid DNA can be distinguished by using selectable markers. Frequently-used selectable markers are the genes encoding aminoglycoside phosphotransferase (APH; *neo*$^R$ gene) or hygromycin B phosphotransferase (HPH). Other selectable markers are the genes encoding adenosine deaminase (ADA), dihydrofolate reductase (DHFR), thymidine kinase (TK) or xanthine-guanine phosphoribosyl tranferase (XGPRT; *gpt* gene).

## Primary cells and cell lines

Depending on their origin, cell cultures or cell lines grow as an adherent monolayer or in suspension. Cells or cell lines vary greatly with respect to their growth behavior and nutritional requirements (2). Optimization of cell culture technique is therefore necessary to ensure that cells are healthy and in optimal condition for transfection. For extensive information on culturing of cells, please refer to the manual *Culture of Animal Cells* (2).

### Adherent cells

Adherent cells are anchorage-dependent and propagate as a monolayer attached to the culture vessel. This attachment is essential for proliferation. Most cells derived from tissues are anchorage-dependent with the exception of hemopoietic cells (cells derived from blood).

### Suspension cells

Suspension cells are able to survive and proliferate without attachment. Hemopoietic cells, transformed cell lines, and cells from malignant tumors can be grown in suspension.

### Primary cell culture

Primary cell cultures arise from the outgrowth of migrating cells from a piece of tissue or by enzymatic, chemical, or mechanical dispersal of the tissue. Primary cell cultures are morphologically most similar to the parent tissue.

### Finite cell line

Finite cell lines are formed after the first subculturing (passaging) of a primary cell culture, and can be propagated and subcultured several times.

### Continuous cell line

There is a limit to the number of generations that a finite cell line can be propagated. After that it will either die out or acquire a stable, heritable alteration, giving rise to a continuous cell line. This alteration is commonly known as in vitro transformation or immortalization, and frequently correlates with tumorigenicity.

## Transfection Considerations

### Media and supplements

Media are composed of a mixture of essential salts, nutrients, and buffering agents. Sterile media are usually purchased in solution. Alternatively, packaged premixed powders are available. Most media purchased are guaranteed to be mycoplasma-and endotoxin-free. Supplements to the media must include glutamine and can include nonessential amino acids, sodium pyruvate, and antibiotics. Some common media include DMEM, F12, DMEM/F12, RPMI 1640, MEM, and S-MEM.

### Serum

In most cases media are supplemented shortly before use with serum. Fetal calf serum (FCS) is often used, but for some applications less expensive sera such as horse or calf serum can be used. Generally, serum is a partially undefined material, which contains growth- and attachment factors and may show considerable variation in the ability to support growth of particular cells. Variations in the serum quality can also lead to variation in transfection efficiency. In general, it is advisable to test a small lot of serum from a reputable supplier with a control cell line and assay before performing transfection experiments. Once a given lot has been shown to yield satisfactory and reproducible results, additional sera from the same lot should be purchased.

### Transfection methods

Of the variety of different transfection methods described in the literature (3, 4), the DEAE-dextran method, the calcium-phosphate method, electroporation, and liposome-mediated transfection are the most commonly used. Each individual method has its characteristic advantages and disadvantages and the choice of transfection method strongly influences transfection results. SuperFect Transfection Reagent represents a completely new class of activated-dendrimer transfection reagent, and has been designed to offer very high transfection efficiencies and reproducibility, while minimizing cytotoxic effects.

### Plasmid DNA quality

Plasmid DNA quality strongly influences several transfection parameters such as efficiency, reproducibility, and toxicity, as well as interpretation of results. Therefore only plasmid DNA of the highest quality, which is completely free of contaminating RNA, genomic DNA, and proteins should be used. DNA purified using HiSpeed, QIAfilter, and QIAGEN Plasmid Kits is well suited for transfection of most cell lines. For highest reproducibility and best results with all cell lines, we recommend DNA purified using the EndoFree Plasmid Kit. This kit quickly and efficiently removes bacterial endotoxins during the plasmid purification procedure, ensuring optimal transfection results.

## Genetic reporter systems

After cloning a gene of interest, transfection is a useful tool to determine how *cis*-acting sequences, such as promoters and enhancers, and *trans*-acting factors, such as transcription factors, act together to control eukaryotic gene expression. Common methods to monitor gene expression involve techniques such as northern blot analysis or nuclease protection assays to quantitate the specific mRNAs transcribed from the gene of interest. Since these procedures are time-consuming and inconvenient for multiple samples (resulting from multiple constructs), an alternative approach is to link the presumed *cis*-acting sequences from the gene of interest to the coding sequence of an unrelated reporter gene (see

examples below) (3, 4). The reporter gene provides an indirect way of measuring how such regulatory sequences influence gene expression. Reporter genes are also useful in serving as controls. Transfection efficiencies between transfection experiments can be standardized by comparing the expression of the reporter gene product. Further information on genetic reporter systems can be obtained from current molecular biology manuals (3, 4).

In choosing a suitable reporter system, several considerations should be taken into account. First, the reporter gene should be absent from the cells used in the study or easily distinguished from the native form of the gene. Second, the assay for the reporter gene product should be quick, easy, sensitive, and inexpensive. In particular, a broad linear range is important to enable detection of both small and large changes in the reporter gene expression. Finally, the presence of the reporter gene should not affect the physiology of the cells being used.

## Commonly used reporters

### Chloramphenicol acetyltransferase

The prokaryotic enzyme chloramphenicol acetyltransferase (CAT) is commonly used as a reporter. This enzyme catalyzes the transfer of acetyl groups from acetyl coenzyme A to chloramphenicol. In the common CAT assay, cell lysates prepared from transfected cells are incubated with $^{14}$C-labeled chloramphenicol. The resulting acetylated and unacetylated forms of chloramphenicol are separated by thin-layer chromatography. A qualitative estimate of CAT activity can be obtained simply by exposing the plates to X-ray film. For quantitative analysis, the separated bands can be scraped from the thin-layer plate and the levels of radioactivity measured in a scintillation counter. Currently, a CAT ELISA is also often used. In this assay the **total expression** of the chloramphenicol acetyltransferase is measured via antibody detection, in contrast to the classic CAT assay described above, which determines only the **active protein**.

### Firefly luciferase

Luciferase catalyses a bioluminescent reaction involving the substrate luciferin, ATP, $Mg^{2+}$, and molecular oxygen. When these components are mixed with cell lysates containing luciferase, a flash of light is emitted. Light signals are detected using a luminometer or a liquid scintillation counter.

### β-galactosidase

The prokaryotic enzyme β-galactosidase can be assayed colorimetrically using the substrate *o*-nitrophenyl-β-D-galactopyranoside (ONPG). The hydrolysis of ONPG by β-galactosidase yields a yellow-colored product, *o*-nitrophenol, which can be measured photometrically.

### Human growth hormone (hGH)

The assay for human growth hormone is based on immunological detection of hGH secreted by transfected cells. Specific $^{125}$I-labeled antibodies against hGH are used and results are monitored in a scintillation counter. Currently, a sandwich-ELISA is also often used, which involves an antibody coupled ELISA plate. The hGH protein binds to the antibody on the plate, a digoxygenated antibody binds to hGH, and a secondary antibody coupled to alkaline phosphatase is used for detection.

### Green fluorescent protein

Green fluorescent protein (GFP), originally isolated from the jellyfish *Aequorea victoria* (3), has the ability to absorb blue light and emit green light. This unique protein can be expressed in mammalian cells and protein expression can be visually monitored in living cells. Although the system provides a convenient way to detect protein expression without a specific assay, quantitative analysis is limited. This reporter gene system is best suited for in situ detection of gene expression, such as localization studies of fusion proteins within cells.

## References

For a complete list of references, visit the QIAGEN Reference Database online at **www.qiagen.com/RefDB/search.asp** or contact QIAGEN Technical Services or you local distributor.


1. *Tang, M.X., Redemann, C.T. and Szoka, Jr., F.C. (1996) In vitro gene delivery by degraded polyamidoamine dendrimers. Bioconjugate Chem.* ***7****, 703.*

2. *Freshney, R.I.,* Culture of Animal Cells, A Manual of Basic Technique, *3rd Ed., Wiley-Liss.*

3. *Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K., eds. (1991)* Current Protocols in Molecular Biology, *New York: Wiley Interscience.*

4. *Sambrook, J., Fritsch, E.F., and Maniatis, T., eds. (1989)* Molecular cloning — a laboratory manual, *2nd Ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.*

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