Effectene® Transfection Reagent Handbook

The next generation in lipid technology

May 2002

QIAGEN

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Contents

Kit Contents

1 ml Effectene® Transfection Reagent (1 mg/ml), 0.8 ml Enhancer (1 mg/ml), 2 x 15 ml Buffer EC, sufficient for 40 transfections in 60 mm dishes, or 160 transfections in 12-well plates, following the standard protocol

or

4 x 1 ml Effectene Transfection Reagent (1 mg/ml), 4 x 0.8 ml Enhancer (1 mg/ml), 8 x 15 ml Buffer EC, sufficient for 160 transfections in 60 mm dishes or 640 transfections in12-well plates, following the standard protocol.

Storage and Stability

Effectene Transfection Reagent, Enhancer, and Buffer EC are supplied as ready-to-use solutions and are shipped at ambient temperature without loss in stability. However, they should be stored at 2–8°C upon arrival. All components are stable for 1 year at 2–8°C. In contrast to many liposome-based reagents, Effectene Reagent is not sensitive to oxygen so it does not require storage under an inert gas. Additionally, Effectene Reagent does not need to be stored on ice during the transfection procedure.

Quality Control

Endotoxin levels are <10 EU/ml as determined using a Kinetic-QCL test (BioWhittaker, Inc).

Effectene Transfection Reagent is tested by transfection of plasmid pCMVβ into HeLaS3 and COS-7 cells to ensure lot-to-lot consistency. Microbial limit tests guarantee the absence of any contaminating bacteria or fungi.

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 Effectene Transfection Reagent or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are 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 encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For details on other products for transfection available from QIAGEN, please contact your local QIAGEN Technical Service Department or QIAGEN distributor (see inside front cover).

Product Use Limitations

Effectene Transfection Reagent is developed, designed, and sold for research purposes only. It is not to be used for human diagnostic or drug purposes or to be administered to humans 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).

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.

Introduction

Effectene Transfection Reagent is a unique non-liposomal lipid formulation designed to achieve high transfection efficiencies. Effectene Transfection Reagent offers significant advantages over many liposome-based reagents and other transfection methods.

Effectene Transfection Reagent means:

- Fast and easy transfection — no transfection-complex removal needed for most cell types
- Minimal cytotoxicity by transfecting in the presence of serum — highly suited for primary cells or sensitive cell lines
- High transfection efficiencies with a wide variety of cell lines; particularly good for primary cells
- Significantly less DNA required — typically only one-fifth of that needed for most liposome-based reagents

The Effectene principle

Effectene Reagent is used in conjunction with the Enhancer and the DNA-condensation buffer (Buffer EC) to achieve high transfection efficiencies. In the first step of Effectene–DNA complex formation, the DNA is condensed by interaction with the Enhancer in a defined buffer system. Effectene Reagent is then added to the condensed DNA to produce condensed Effectene–DNA complexes. The Effectene–DNA complexes are mixed with medium and directly added to the cells.

Effectene Reagent spontaneously forms micelle structures that show no size or batch variation, as found with preformulated liposome reagents. This unique feature ensures excellent reproducibility of transfection complex formation. The process of highly condensing DNA molecules and then coating them with Effectene Reagent is a particularly effective way to transfer DNA into eukaryotic cells.

Broad cell line spectrum

Effectene Transfection Reagent has been used for transfection of a variety of different cell lines and primary cells, and yields significantly better transfection results than many widely used liposome-based transfection reagents. A searchable list of cell lines and primary cells successfully transfected using Effectene Reagent, as well as customer-developed protocols, is available at the Transfection Tools web site — **www.qiagen.com/transfectiontools/**. In addition, a searchable online literature database is available at **www.qiagen.com/literature/**.

General Guidelines

Transfection efficiencies are influenced 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 does not become altered. Highest transfection efficiencies are obtained using the confluence levels indicated in the appropriate protocol sections. We also 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 Effectene Reagent, and during subsequent incubation for gene expression.

Effect of serum

In contrast to many liposome-based transfection reagents, Effectene Reagent enables transfection in the presence of serum without lowering transfection efficiencies. Therefore, serum can be included in medium when incubating cells with transfection complexes (step 8 of protocols), and during subsequent incubation for gene expression. Use the same percentage of serum that cells have been adapted to in culture.

Vector construct

The type of transfection vector (plasmid DNA, RNA, 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 results in lower DNA uptake by the cells, relative to supercoiled DNA, but yields optimal integration of DNA into the host genome.

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 13–16 yielded high transfection levels for many cell lines tested. 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 Effectene Reagent, DNA, and Effectene–DNA complex; the cell number/confluency prior to transfection; and the length of exposure of cells to Effectene–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

Table 1 lists the recommended number of adherent cells to seed per culture plate/dish **the day before transfection**, and the recommended number of suspension cells to seed and the volume of medium to use **the day of transfection**. **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. **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. 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 x 10^4	0.5–2.0 x 10^5	100
48-well plate	1.0–4.0 x 10^4	1.0–3.5 x 10^5	150
24-well plate	2.0–8.0 x 10^4	2.0–7.0 x 10^5	350
12-well plate	0.4–2.0 x 10^5	0.5–1.5 x 10^6	800
6-well plate	0.9–4.0 x 10^5	1.0–3.5 x 10^6	1600
60 mm dish	2.0–8.0 x 10^5	2.5–7.5 x 10^6	4000
100 mm dish	0.5–2.5 x 10^6	0.5–2.0 x 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. When seeding adherent cells, use a volume of medium suitable for your cell culture format.

Amount of DNA

The optimal quantity of plasmid DNA used for transfection is determined by the properties of the transfected plasmid, and 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, gene expression 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 Effectene Reagent in 60 mm dishes is 0.5–2 µg. Although this DNA quantity may seem low, 1 µg DNA per 60 mm dish is usually sufficient for transfection.**

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 16, respectively.

Amount of Enhancer

The ratios of DNA to Enhancer provided in the protocols on pages 13–16 should not be changed. The ratio of DNA to Enhancer is 1 µg DNA to 8 µl Enhancer. Efficient condensation of DNA with Enhancer is determined only by the mass quantity of DNA. (Cell type and plasmid size do not influence the DNA-to-Enhancer ratio).

Ratio of Effectene Reagent to DNA–Enhancer mixture

The overall charge of the Effectene–DNA complex is determined by the ratio of Effectene Reagent to DNA–Enhancer mixture. Optimal binding of Effectene–DNA complexes to negatively charged groups (e.g. sialylated glycoproteins) on the cell surface requires a slightly net positive charge. The ratio of Effectene Reagent (µl) to DNA (µg) is an important factor to optimize for every new cell line and DNA construct used. As a starting point for optimization we recommend using a DNA:Effectene ratio of 1 µg DNA to 25 µl Effectene Reagent in 60 mm dishes.

A pipetting scheme for optimizing transfection of adherent and suspension cells in 60 mm dishes is provided in Table 2. To optimize transfection in other culture formats, prepare separate transfection mixtures: 1) use the starting point DNA and Effectene Reagent quantities listed in Tables 3 and 4 (pages 14 and 16, respectively), 2) use roughly half the listed ratios and quantities, and 3) use double the listed ratios and quantities.

Removal of Effectene–DNA complexes

Removal of transfection complexes in many cases is not necessary. However, if cytotoxicity is observed, remove the Effectene–DNA complexes 6–18 hours after their addition to the cells, wash the cells with PBS, and add fresh growth medium.

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

DNA (µg)	Ratio of DNA to Effectene Reagent 1:10	1:25	1:50
0.5	0.5 µg DNA 4.0 µl Enhancer 5.0 µl Effectene Reagent	0.5 µg DNA 4.0 µl Enhancer 12.5 µl Effectene Reagent	0.5 µg DNA 4.0 µl Enhancer 25.0 µl Effectene Reagent
1.0	1 µg DNA 8 µl Enhancer 10 µl Effectene Reagent	1 µg DNA 8 µl Enhancer 25 µl Effectene Reagent	1 µg DNA 8 µl Enhancer 50 µl Effectene Reagent
2.0	2 µg DNA 16 µl Enhancer 20 µl Effectene Reagent	2 µg DNA 16 µl Enhancer 50 µl Effectene Reagent	2 µg DNA 16 µl Enhancer 100 µl Effectene Reagent

High-throughput transfection

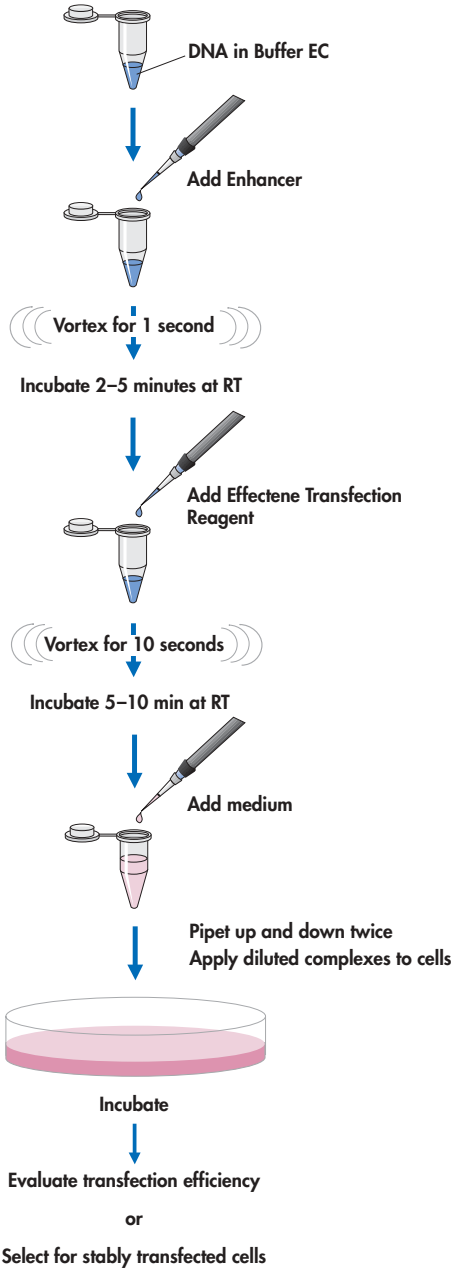
QIAGEN has developed rapid transfection protocols for COS-7, NIH/3T3, HeLa, HeLa-S3, 293, and CHO cells in 96-well plates using Effectene Transfection Reagent. These optimized, cell-specific protocols allow same-day plating and transfection of cells, and are highly suited for high-throughput transfection experiments. Copies of the protocols are available on request from QIAGEN Technical Service Departments (see inside front cover).

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.

Transfection Procedure for Effectene Transfection Reagent



Protocol for Transient or Stable Transfection of Adherent Cells

The following protocol is for transfection of adherent cells in 60 mm dishes. As a starting point, use 1 µg DNA per 60 mm dish. **Although this DNA quantity may seem low, it is usually sufficient for transfection.** Starting points for optimizing transfection in other formats are listed in Table 3 on page 14. See Table 1 on page 9 for the recommended number of cells to seed. Optimal transfection conditions should be determined for every cell line if the highest transfection efficiency with Effectene Reagent is required. Please refer to the optimization guidelines on pages 9–11.

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 containing serum and antibiotics.**
2. **Incubate the cells under their normal growth conditions (generally 37°C and 5% CO_2). The dishes should be 40–80% confluent on the day of transfection.**
3. **The day of transfection, dilute 1 µg DNA dissolved in TE buffer, pH 7 to pH 8 (minimum DNA concentration: 0.1 µg/µl) with the DNA-condensation buffer, Buffer EC, to a total volume of 150 µl. Add 8 µl Enhancer and mix by vortexing for 1 s.**

   IMPORTANT: Always keep the ratio of DNA to Enhancer constant.

   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. **Incubate at room temperature (15–25°C) for 2–5 min then spin down the mixture for a few seconds to remove drops from the top of the tube.**
5. **Add 25 µl Effectene Transfection Reagent to the DNA-Enhancer mixture. Mix by pipetting up and down 5 times, or by vortexing for 10 s.**

   Note: It is not necessary to keep Effectene 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. **While complex formation takes place, gently aspirate the growth medium from the plate, and wash cells once with 4 ml PBS. Add 4 ml fresh growth medium (can contain serum and antibiotics) to the cells.**
8. **Add 1 ml growth medium (can contain 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 transfection complexes.**

9. **Incubate the cells with the transfection complexes under their normal growth conditions for an appropriate time for expression of the transfected gene. The incubation time is determined by the assay and gene used.**

   Optional: In many cases, removal of transfection complexes is not necessary. However, if cytotoxicity is observed, remove the Effectene–DNA complexes after 6–18 h, wash the cells once with PBS, and add 5 ml fresh growth medium.

10. **For transient transfections, assay cells for expression of the transfected gene.**

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

    For stable transfections, passage cells 1:5 to 1:10 into the appropriate selective medium 24–48 h after transfection. Maintain cells in selective medium 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 growth medium (i.e., with no selective drug) and then incubate them for 1–2 days before addition of selective medium.

Table 3. Starting points for optimizing the transfection of adherent cells in different formats using Effectene Reagent. Volumes given apply to each well of multiwell plates.

Culture format	DNA (µg)	Enhancer (µl)	Final volume of DNA in Buffer EC (µl)	Volume of Effectene Reagent (µl)	Volume of medium to add to cells (µl)†	Volume of medium to add to complexes (µl)†
Protocol step	**3**	**3**	**3**	**5**	**7**	**8**
96-well plate	0.1	0.8	30	2.5*	100	0
48-well plate	0.15	1.2	50	4*	150	200
24-well plate	0.2	1.6	60	5	350	350
12-well plate	0.3	2.4	75	6	800	400
6-well plate	0.4	3.2	100	10	1600	600
60 mm dish	1.0	8.0	150	25	4000	1000
100 mm dish	2.0	16.0	300	60	7000	3000

** If transfections are performed in 96- or 48-well plates, dilute the Effectene Reagent with Buffer EC to a total volume of 20 µl or 50 µl, respectively, before addition to the diluted DNA–Enhancer mixture prepared in step 3.*

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

Protocol for Transient or Stable Transfection of Suspension Cells

The following protocol is for transfection of suspension cells in 60 mm dishes. As a starting point, use 1 µg DNA per 60 mm dish. **Although this DNA quantity may seem low, it is usually sufficient for transfection.** Starting points for optimizing transfection in other formats are listed in Table 4 on page 16. See Table 1 on page 9 for the recommended number of cells to seed and the volume of medium to use in other cell culture formats. Optimal transfection conditions should be determined for every cell line if the highest transfection efficiency with Effectene Reagent is required. Please refer to the optimization guidelines on pages 9–11.

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 growth medium containing serum and antibiotics.**
4. **Dilute 1 µg DNA dissolved in TE buffer, pH 7 to pH 8 (minimum DNA concentration: 0.1 µg/µl) with the DNA-condensation buffer, Buffer EC, to a total volume of 150 µl. Add 8 µl Enhancer and mix by vortexing for 1 s.**

   IMPORTANT: Always keep the ratio of DNA to Enhancer constant.

   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. **Incubate at room temperature (15–25°C) for 2–5 min then spin down the mixture for a few seconds to remove drops from the top of the tube.**
6. **Add 25 µl Effectene Reagent to the DNA–Enhancer solution. Mix by pipetting up and down 5 times, or by vortexing for 10 s.**

   Note: It is not necessary to keep Effectene Reagent on ice at all times. 10–15 min at room temperature will not alter its stability.
7. **Incubate the samples for 5–10 min at room temperature (15–25°C) to allow transfection-complex formation.**
8. **Add 1 ml growth medium (can contain serum and antibiotics) to the tube containing the transfection complexes. Mix by pipetting up and down twice then 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.**

9. **Incubate the cells with the transfection complexes under their normal growth conditions (generally 37°C and 5% CO_2) for an appropriate time for expression of the transfected gene. The incubation time is determined by the assay and gene used.**

   Optional: In many cases, removal of transfection complexes is not necessary. However, if cytotoxicity is observed, centrifuge cells after 6–18 h, remove medium containing the complexes, wash the cells with PBS, then resuspend in 5 ml fresh growth medium.

10. **For transient transfections, assay cells for expression of the transfected gene.**

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

    For stable transfections, 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 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 growth medium (i.e., with no selective drug) and then incubate them for 1–2 days before addition of selective medium.

Table 4. Starting points for optimizing the transfection of suspension cells in different formats using Effectene Reagent. Volumes given apply to each well of multiwell plates.

Culture format	DNA (µg)	Enhancer (µl)	Final volume of DNA in Buffer EC (µl)	Volume of Effectene Reagent (µl)	Volume of medium to add to complexes (µl)†
Protocol step	**4**	**4**	**4**	**6**	**8**
96-well plate	0.1	0.8	30	2.5*	0
48-well plate	0.15	1.2	50	4*	200
24-well plate	0.2	1.6	60	5	350
12-well plate	0.3	2.4	75	6	400
6-well plate	0.4	3.2	100	10	600
60 mm dish	1.0	8.0	150	25	1000
100 mm dish	2.0	16	300	60	3000

** If transfections are performed in 96- or 48-well plates, dilute Effectene Reagent with Buffer EC to a total volume of 20 µl or 50 µl, respectively, before addition to the diluted DNA–Enhancer mixture prepared in step 4.*

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

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.

Observation	Possible Cause	Comments and suggestions
Low transfection efficiency	Effectene Reagent to DNA ratio is sub-optimal	If the ratio of Effectene Reagent to DNA is sub-optimal, 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 Effectene Reagent to DNA ratio according to the optimization section (pages 9–11).
	Insufficient amount of Effectene–DNA complex	If the transfection efficiency is lower than expected and cytotoxicity acceptably low, increase the overall amount of Effectene–DNA complex. See the pipetting scheme on page 11.
	Incubation time for gene expression is sub-optimal	Different cell types achieve maximal expression levels at different times post-transfection. This should be kept in mind when determining the length of incubation after transfection. If the time point of maximal expression is not known for a particular cell line, a time course experiment may be necessary.
	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.
	Cell density at the time of Effectene–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 9).

Observation	Possible cause	Comments and suggestions
	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.
	Reporter assay problem	Include positive controls to ensure that the reporter assay is working properly.
Excessive cell death	Excessive exposure of cells to Effectene–DNA complexes	If sensitive cells (e.g., primary cells) or cell lines demonstrate extensive cell death after treatment with Effectene Reagent, remove the Effectene–DNA complexes after 6–18 h. Wash cells carefully after removing complexes. With sensitive cell lines, we recommend 2–4 careful washing steps with complete medium rather than PBS.
	Amount of Effectene–DNA complexes too high	If cell death continues after reducing exposure times, decrease the amount of Effectene–DNA complexes (see the pipetting scheme on page 11), but keep the ratio of Effectene Reagent to DNA constant.
	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.
	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 the amount of plasmid DNA (see page 10) is recommended for every new plasmid and/or new cell line used.

Observation	Possible cause	Comments and suggestions
Variable transfection efficiencies in replicate experiments	Inconsistent cell confluency in replicate experiments	Count cells prior to seeding to ensure that the same number of cells are seeded for each experiment. Keep incubation times between seeding and complex addition consistent between experiments.
	Possible mycoplasma contamination	Mycoplasma contamination influences transfection efficiency. Variations in the growth behavior of mycoplasma-infected cells will lead to different transfection efficiencies between replicate experiments.
	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).
	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: Buffer Composition

Buffer	Composition	Storage
1x PBS (phosphate-buffered saline)	137 mM NaCl 2.7 mM KCl 4.3 mM Na_2HPO_4 1.47 mM KH_2PO_4 Adjust to a final pH of 7.4	Room temp.
1x TE buffer, pH 7–8	10 mM Tris·Cl, pH 7–8 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 (1, 2).

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 24–96 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 results in lower DNA uptake by the cells relative to supercoiled DNA, it yields optimal integration of DNA into the host genome. 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 (1). Optimization of cell culture technique is 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* (1).

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 hematopoetic cells (cells derived from blood).

Suspension cells

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

Primary cell cultures

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 lines

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 lines

There is a limit to the number of generations that a finite cell line can be propagated. Beyond this limit the finite cell line 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 that contains growth and attachment factors, and may show considerable variation in the ability to support growth of particular cells. Variations in 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 (2, 3), the DEAE-dextran method, the calcium-phosphate method, electroporation, liposome-mediated, and activated-dendrimer–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. Effectene Transfection Reagent represents a completely new class of lipid-based 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 using 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) (2, 3). 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 (2, 3).

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. 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

The assay for human growth hormone (hGH) 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* (2), 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.

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