Transfection Reagent Selector Kit Handbook

For determining the optimal reagent for your cells

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QIAGEN

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Contents

Kit Contents

0.3 ml SuperFect Transfection Reagent (3 mg/ml)

0.3 ml Effectene Transfection Reagent (1 mg/ml)

0.2 ml Enhancer (1 mg/ml)

1x 15 ml Buffer EC

This selector kit enables you to determine the best reagent and optimal transfection conditions for a given cell line/type by testing 9 different transfection conditions and 2 controls for each QIAGEN® Transfection Reagent in 6-well format. If you prefer to use a different culture format for your determination, please contact QIAGEN Technical Services for the appropriate pipetting scheme.

Storage and Stability

SuperFect™ Transfection Reagent, 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, SuperFect Reagent and Effectene Reagent are not sensitive to oxygen so they do not require storage under an inert gas. Additionally, SuperFect Reagent and Effectene Reagent do 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).

SuperFect Transfection Reagent and Effectene Transfection Reagent are tested by transfection of plasmid pCMVβ (CLONTECH) into HeLaS3 and COS-7 cells to ensure lot-to-lot consistency. Sterility 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 SuperFect or 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 one of the QIAGEN Technical Service Departments or your local QIAGEN distributor listed on the last page.

Introduction

The Transfection Reagent Selector Kit contains two new QIAGEN Transfection Reagents, Effectene and SuperFect, which are based on the latest advances in transfection technology. The selector protocol in this handbook is designed to test both reagents and different transfection conditions in parallel in order to determine the optimal reagent and transfection conditions for your cells. The selector kit protocol is designed for 6-well plates. If you prefer to use a different culture format for your determination, please contact QIAGEN Technical Services for the appropriate pipetting scheme.

If you prefer to test or use the QIAGEN Transfection Reagents separately, please contact QIAGEN Technical Services or your local distributor for a copy of the *Effectene Transfection Reagent Handbook* or the *SuperFect Transfection Reagent Handbook*, or visit us at www.qiagen.com/literature/index.html to obtain the handbooks as Adobe™ PDF files. Whatever your transfection needs, QIAGEN has the answer.

SuperFect Transfection Reagent

SuperFect Transfection Reagent represents a new class of activated-dendrimer transfection reagent designed for outstanding transfection results (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 that allows them to bind to negatively charged receptors (e.g. sialylated glycoproteins) on the surface of eukaryotic cells. Once inside the cell, SuperFect Reagent buffers the lysosome after it has fused with the endosome, leading to pH inhibition of lysosomal nucleases. This ensures stability of SuperFect–DNA complexes and the transport of intact DNA to the nucleus.

Effectene Transfection Reagent

Effectene Transfection Reagent is based on a proprietary non-liposomal lipid. Effectene Reagent works together with a specific DNA-condensing Enhancer to produce outstanding 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.

SuperFect Reagent and Effectene Reagent yield significantly better transfection results than many widely used liposome transfection reagents. SuperFect and Effectene Reagent are suitable for efficient transfection of a wide variety of cell lines (Table 1, page 8). Effectene Reagent is also the reagent of choice for primary cells. SuperFect Reagent and Effectene Reagent have been designed to efficiently transfect cells in the presence of serum, thereby reducing stress on the cells and lowering cytotoxicity. In addition, Effectene Reagent requires significantly less DNA to obtain high levels of transfection than do many liposome-based reagents and other transfection methods.

SuperFect Reagent and Effectene Reagent both offer:

- Outstanding transfection efficiencies for many cell lines
- Excellent reproducibility
- Decreased cytotoxicity
- Transfection in the presence of serum

Additional features of Effectene Transfection Reagent:

- Particularly suitable for primary cells
- Significantly less DNA required for efficient DNA uptake
- No transfection complex removal needed for most cell lines

QIAGEN has the right transfection reagent for your application

The table below lists examples of cells that have been successfully transfected with one or both reagents. These guidelines for reagent choice are based on our current knowledge from tests performed so far.

Table 1. Examples of cells successfully transfected with Effectene Reagent and SuperFect Reagent

Cell line	Cell source and type		Origin	Effectene Reagent	SuperFect Reagent
Adherent cells					
HeLa S3	Cervix carcinoma	Epithelial	Human	✓	✓
293	Kidney transformed	Epithelial	Human	✓	✓
Saos-2	Osteosarcoma	Epithelial	Human	✓	✓
HT-1080	Fibrosarcoma	Epithelial	Human	n.d.	✓
Huh7	Hepatoma	Epithelial	Human	✓	✓
LMH	Hepatoma	Epithelial	Chicken	✓	✓
PTC	Kidney tubular	Epithelial	Mouse	✓	✓
HT-29	Colon adenocarcinoma	Epithelial	Human	✓	✓
SW-480	Colon adenocarcinoma	Epithelial	Human	✓	✓
ECV304	Umbilical cord	Endothelial	Human	✓	✓
HaCat	Keratinocyte		Human	✓	✓
HTZ-19	Melanoma		Human	✓	n.d.
COS-7	Kidney, SV40-transformed	Fibroblast	Monkey	✓	✓
NIH/3T3	Embryo	Fibroblast	Mouse	✓	✓
CRE BAG2	MMLV-transfected	Fibroblast	Mouse	n.d.	✓
PA 317	Embryo	Fibroblast	Mouse	n.d.	✓
AtT20	Pituitary tumor		Mouse	✓	✓
L6	Skeletal muscle	Myoblast	Rat	✓	✓
Suspension cells					
Jurkat	T-cell leukemia	Lymphoblast	Human	✓	✓
HUT78	T-cell leukemia		Human	n.d.	✓
U937	Histiocytic lymphoma	Lymphoblast	Human	✓	✓
MOLT-4	T-cell leukemia	Lymphoblast	Human	✓	✓
THP-1	Monocyte		Human	✓	X
M12.4	B cell	Lymphoblast	Mouse	✓	✓
MC-9	Mast cell		Mouse	✓	✓
SK-MEL-1	Malignant melanoma		Human	✓	✓
Primary cells					
Primary		Umbilical vein endothelial cells	Human	✓	✓
Primary		Fibroblasts	Mouse	✓	✓
Primary		Bone marrow cells	Mouse	✓	✓
Primary		Hepatocytes	Rat	✓	n.d.
Primary		Aortic smooth muscle cells	Rabbit	✓	n.d.
Primary		Gastric parietal cells	Rabbit	✓	✓

Blue *denotes cell lines that were evaluated at laboratories at QIAGEN, and* ***black*** *denotes cell lines that were evaluated by independent laboratories.*

✓ *represents successful transfection with the indicated reagent.*

indicates the recommended reagent based on our current knowledge.

X *indicates that the reagent did not perform well with this cell line for the plasmid construct tested.*

n.d.*: not determined for this cell line.*

General Guidelines

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 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 h before transfection. This provides normal cell metabolism and increases the likelihood of DNA uptake. Microbial contamination, for example with bacteria, 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 Effectene Reagent, and during subsequent incubation for gene expression.

Effect of serum

In contrast to many liposome transfection reagents, SuperFect Reagent and Effectene Reagent enable transfection in the presence of serum without lowering transfection efficiencies. Therefore, serum can be included in medium when incubating cells with transfection complexes 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.

DNA quality

The best results are achieved when plasmid DNA of the highest purity is used for transfection. DNA purified with QIAGEN and QIAfilter™ Plasmid Kits is ideally suited for transfection of most cell lines. For transfection of endotoxin-sensitive cells, we recommend using DNA purified with EndoFree™ Plasmid Kits. These kits efficiently remove bacterial lipopolysaccharide molecules during the plasmid purification procedure, ensuring optimal transfection results.

Selector Protocol for Effectene Reagent and SuperFect Reagent

QIAGEN has developed the following protocol for testing Effectene Reagent and SuperFect Reagent in parallel in 6-well plates. The selector kit includes quantities of each QIAGEN Transfection Reagent sufficient to perform two sets of optimization experiments as described. For transfection in other culture formats, please contact QIAGEN Technical Services for the appropriate pipetting scheme.

This selector protocol enables you to determine the best reagent and optimal transfection conditions for your cells by testing 9 different transfection conditions for each QIAGEN Transfection Reagent. The transfection mixes contain different amounts of DNA and DNA–Transfection Reagent complex, and different ratios of DNA to Transfection Reagent. The selector protocol procedure is illustrated schematically in Figure 1 and the corresponding pipetting scheme is provided in Figure 2. Two controls for each QIAGEN Transfection Reagent are also included in the protocol. The Reagent-but-no-DNA control checks whether the reagent was toxic to the cells, and the no-DNA-no-reagent control checks whether the cell culture conditions were correct. The reagent and transfection conditions yielding the highest transfection efficiency should be used for future experiments using the same cell line/plasmid DNA combination. In this protocol, two 6-well plates are used for testing each QIAGEN Transfection Reagent.

Optimal transfection conditions should be determined for every cell line if the highest transfection efficiency with either QIAGEN Transfection Reagent is required. For additional information on optimization of transfection efficiencies, please refer to the optimization guidelines on pages 16–17. 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.

If you prefer to test or use the QIAGEN Transfection Reagents separately, please contact QIAGEN Technical Services or your local distributor for a copy of the *Effectene Transfection Reagent Handbook* or the *SuperFect Transfection Reagent Handbook*, or visit us at www.qiagen.com/literature/index.html to obtain the handbooks as Adobe™ PDF files.

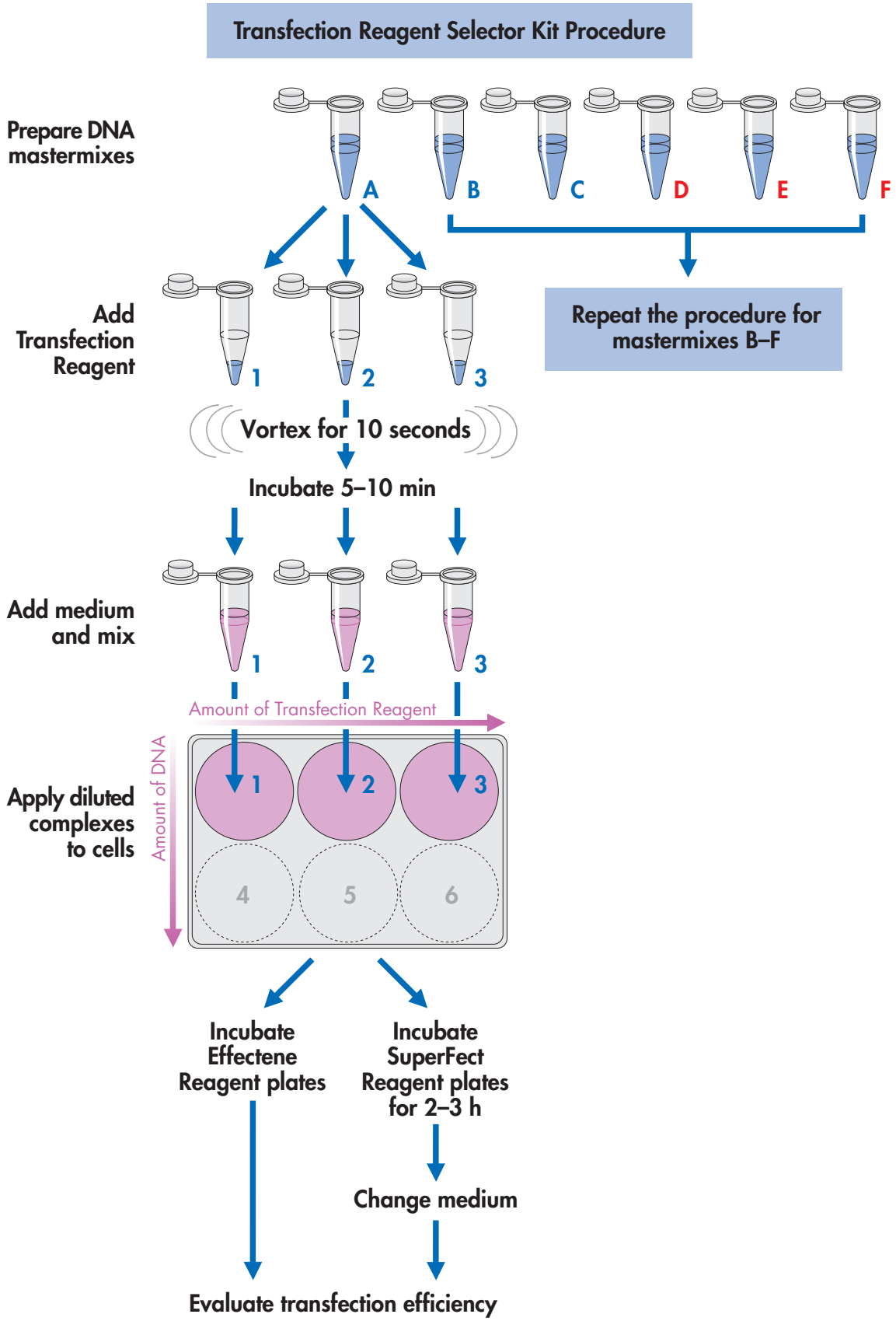

Figure 1. *Procedure for Transfection Reagent Selector Kit.*

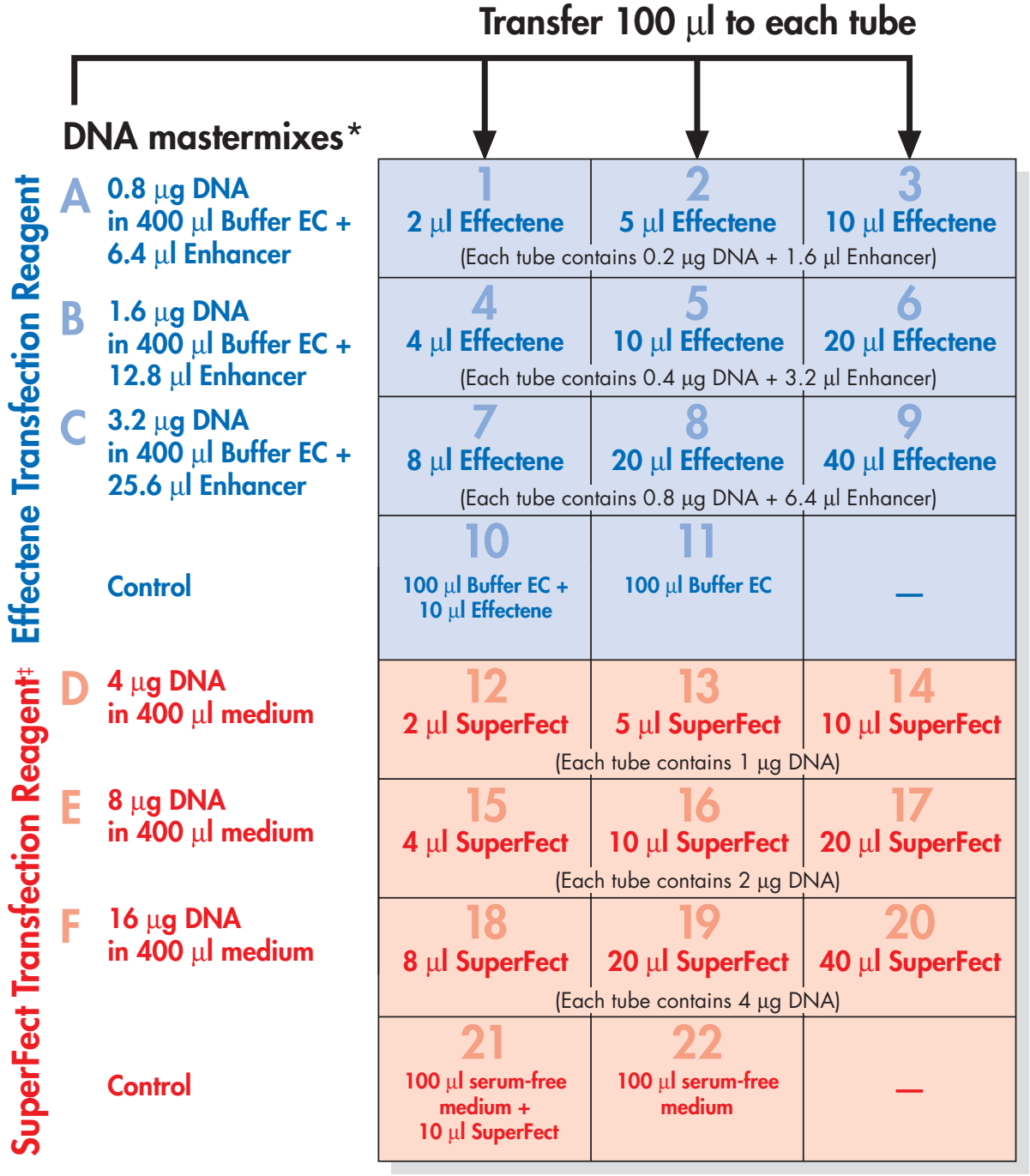

Figure 2. *Pipetting scheme for selector protocol with adherent cells in 6-well plates†*

* *It is important to prepare mastermixes A–C in the order specified, since direct mixing of the DNA with the Enhancer may lead to precipitation.*

† *If you prefer to use a different culture format for your determination, please contact QIAGEN Technical Services for the appropriate pipetting scheme.*

NOTE: For transfection of suspension cells in 6-well format, better results may be obtained by using approximately 80% of the SuperFect Reagent volumes indicated in Figure 2.

Cell-culture preparation

1. **Prepare two 6-well plates for Effectene Reagent and label the wells 1 –11. Prepare two 6-well plates for SuperFect Reagent and label the wells 12–22. The day before transfection, seed 0.9– 4.0 x 10^5 cells (depending on the cell type) per well in 2 ml of appropriate growth medium containing serum and antibiotics. (For suspension cells, seed 1.0–3.5 x 10^6 cells in 1.6 ml of medium on the day of transfection.)**
2. **Incubate cells at 37°C and 5% CO_2 in an incubator. The cells should be 40–80% confluent on the day of transfection.**

   Before transfection we recommend to:

   - Check the cells — do they look healthy?
   - Check and note the time between seeding and transfection — this should be kept constant in future experiments.
   - Check and note the confluency of the cells — this should be kept constant in future experiments.
3. **Label twenty-two 1.5-ml microfuge tubes 1–11 for Effectene Reagent and 12–22 for SuperFect Reagent.**
4. **Label six 1.5-ml microfuge tubes A–F for mastermix preparation: A–C for DNA-Enhancer mastermix preparation for Effectene Reagent and D–F for DNA mastermix preparation for SuperFect Reagent.**

Transfection-complex formation

The following steps for complex formation refer to the pipetting scheme outlined in Figure 2 on page 12.

5. **DNA-Enhancer mastermixes for Effectene Reagent**

   IMPORTANT: It is important to prepare mastermixes A–C in the order specified, since direct mixing of the DNA with the Enhancer may lead to precipitation.

   Mastermix A: Dilute 0.8 µg of DNA in Buffer EC and add 6.4 µl of Enhancer. The final volume should be 400 µl.

   Mastermix B: Dilute 1.6 µg of DNA in Buffer EC and add 12.8 µl of Enhancer. The final volume should be 400 µl.

   Mastermix C: Dilute 3.2 µg of DNA in Buffer EC and add 25.6 µl of Enhancer. The final volume should be 400 µl.

   Mix mastermix tubes A–C by vortexing for 1 second. Incubate at room temperature (20–25°C) for 2–5 min and spin down the mixture for a few seconds to remove drops from the top of the tube.

6. Pipet 100 µl of mastermix A into tubes 1, 2, 3, each of which will finally contain 0.2 µg of DNA.

   Pipet 100 µl of mastermix B into tubes 4, 5, 6, each of which will finally contain 0.4 µg of DNA.

   Pipet 100 µl of mastermix C into tubes 7, 8, 9, each of which will finally contain 0.8 µg of DNA.

7. Controls for Effectene Reagent

   Reagent-but-no-DNA control: Pipet 100 µl of Buffer EC into tube 10.

   No-DNA-no-reagent control: Pipet 100 µl of Buffer EC into tube 11.

8. DNA mastermixes for SuperFect Reagent

   Mastermix D: Dilute 4 µg of DNA in 400 µl of cell growth medium without serum and antibiotics.

   Mastermix E: Dilute 8 µg of DNA in 400 µl of cell growth medium without serum and antibiotics.

   Mastermix F: Dilute 16 µg of DNA in 400 µl of cell growth medium without serum and antibiotics.

   Mix mastermix tubes D–F by vortexing for 1 second. Incubate at room temperature (20–25°C) for 2–5 min and spin down the mixture for a few seconds to remove drops from the top of the tube.

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

9. Pipet 100 µl of mastermix D into tubes 12, 13, 14, each of which will finally contain 1 µg of DNA.

   Pipet 100 µl of mastermix E into tubes 15, 16, 17, each of which will finally contain 2 µg of DNA.

   Pipet 100 µl of mastermix F into tubes 18, 19, 20, each of which will finally contain 4 µg of DNA.

10. Controls for SuperFect Reagent

    Reagent-but-no-DNA control: Pipet 100 µl of growth medium containing no serum or antibiotics into tube 21.

    No-DNA-no-reagent control: Pipet 100 µl of growth medium containing no serum or antibiotics into tube 22.

11. To tubes 1–9 and control tube 10: add the volume of Effectene Reagent specified in Figure 2 on page 12 to the condensed DNA solution. Mix by pipetting up and down 5 times, or by vortexing for 10 seconds.

    Note: It is not necessary to keep Effectene Reagent on ice at all times. 10–15 minutes at room temperature will not alter its stability.

12. **To tubes 12–20 and control tube 21: add the volume of SuperFect Reagent specified in Figure 2 on page 12 to the DNA solutions. Mix by pipetting up and down 5 times, or by vortexing for 10 seconds.**

    Note: It is not necessary to keep SuperFect Reagent on ice at all times. 10–15 minutes at room temperature will not alter its stability.

13. **Incubate all tubes for 5–10 min at room temperature (20–25°C) to allow complex formation.**

14. **For adherent cells, while complex formation takes place gently aspirate the growth medium from all wells and wash cells once with 2 ml PBS. To wells 1–11 add 1600 µl fresh growth medium (can contain serum and antibiotics). To wells 12–22 no medium should be added. For suspension cells, skip this step.**

15. **Add 600 µl of growth medium (can contain serum and antibiotics) to all tubes. Mix by pipetting up and down twice, and immediately add the total contents drop-wise onto the cells in the appropriate wells. Add the contents from tube 1 to well 1, from tube 2 to well 2, from tube 3 to well 3, and so on. Gently swirl each dish to ensure uniform distribution of the complexes.**

16. **Incubate both Effectene Reagent plates (wells 1–11) at 37°C and 5% CO_2 to allow for gene expression.**

    Incubation length is determined by the assay and gene used. Experiments have shown that in many cases removal of transfection complexes is not necessary.

17. **Incubate both SuperFect Reagent plates (wells 12–22) for 2–3 h at 37°C and 5% CO_2 to allow for uptake of the transfection complexes. After this time, remove the medium containing the remaining complexes from the cells by gentle aspiration, and wash cells 1x with 2 ml of PBS. Add fresh growth medium (can contain serum and antibiotics). For suspension cells, removal of transfection complexes is usually not necessary.**

    If cytotoxicity is observed with suspension cells, remove the transfection complexes by centrifugation after a 2–3 hour incubation period. Remove the medium from the cells, resuspend cells in fresh medium containing serum and antibiotics, and continue incubation for an appropriate time for gene expression.

    Post-transfection we recommend to:

    - Check the cells before evaluation of gene expression — is any toxicity apparent?
    - Check and note the incubation time before harvesting for evaluation of gene expression.

18. **To assay for gene expression, proceed as follows:**

    For transient transfection, harvest cells and evaluate 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 expression levels of the reporter gene.

Transfection Optimization

The selector protocol in this handbook enables you to optimize the amount of DNA and Transfection Reagent, and the ratio of DNA to Transfection Reagent for each QIAGEN Transfection Reagent. In addition to these parameters, it is recommended to optimize a number of other parameters in order to achieve optimal transfection efficiency for every new cell line/plasmid DNA combination used. These additional parameters are the cell confluency/density at the time of complex addition and the length of exposure of cells to Transfection Reagent–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 6-well plates, we recommend to seed 0.9–4.0 x 10^5 adherent cells in 2 ml of medium per well **the day prior** to transfection, and 1.0–3.5 x 10^6 suspension cells in 1.6 ml of medium **on the day** of transfection. The actual number of cells depends on cell type and size. If you prefer to use a different culture format for your determination, please contact QIAGEN Technical Services for the recommended number of adherent or suspension cells to seed.

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 prior to 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.

Amount of DNA

The optimal quantity of plasmid DNA used for transfection is determined by the properties of the transfected plasmid which include: 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. A pipetting scheme for optimizing the amount of DNA for transfection in 6-well plates is provided in Figure 2 on page 12.

Amount of Enhancer (Effectene Reagent only)

The ratios of DNA to Enhancer provided in the selector protocol on pages 10–15 should not be changed. The ratio of DNA to Enhancer is 1 µg of DNA to 8 µl of Enhancer. Efficient condensation of DNA with Enhancer is only determined by the mass quantity of DNA. (Cell line or plasmid size do not influence the DNA-to-Enhancer ratio).

Ratio of Transfection Reagent to DNA

The ratio of Transfection Reagent (µl) to DNA (µg) is an important factor to optimize for every new cell line and DNA construct used. Optimal binding of Transfection Reagent–DNA complexes to the negatively charged groups (e.g. sialylated glycoproteins) on the cell surface requires a slightly net positive charge. For Effectene Reagent, the overall charge of the Transfection Reagent–DNA complex is determined by the ratio of Transfection Reagent to DNA–Enhancer mixture. For SuperFect Reagent, the overall charge of the Transfection Reagent–DNA complex is determined by the ratio of Transfection Reagent to DNA. Figure 2 on page 12 represents a pipetting scheme for optimizing the ratio of DNA to Transfection Reagent for transfection of adherent cells or suspension cells in 6-well plates.

Incubation period with Transfection Reagent–DNA complexes

For SuperFect Reagent:

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 h. Optimal results are typically obtained by choosing periods of 2–3 h.

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.

For Effectene Reagent:

For adherent and suspension cells, results show that in many cases, removal of transfection complexes is not necessary. However, if cytotoxicity is observed, remove the Effectene–DNA complexes 6–18 h after addition, wash with PBS, and replace with fresh medium (containing serum and antibiotics), and incubate for gene expression.

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	Transfection Reagent to DNA ratio is sub-optimal	If the ratio of Transfection 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 Transfection Reagent to DNA ratio according to the optimization section (pages 16–17).
	Insufficient amount of Transfection Reagent –DNA complex	If the transfection efficiency is lower than expected and cytotoxicity acceptably low, increase the overall amount of Transfection Reagent–DNA complex. See the example pipetting scheme on page 12.
	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 Transfection Reagent–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 16).

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 QIAGEN or QIAfilter 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 Transfection Reagent–DNA complexes	For Effectene: 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 hours. Wash cells carefully after removing complexes. With sensitive cell lines, we recommend 2–4 careful washing steps with complete medium rather than PBS. For SuperFect: Most adherent cell lines yield optimal results when incubated with SuperFect DNA complexes for 2–3 h. 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 h. 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.

Observation	Possible Cause	Comments and Suggestions
	Amount of Transfection Reagent–DNA complexes too high	If cell death continues after reducing exposure times, decrease the amount of Transfection Reagent– DNA complexes (see pipetting scheme on page 12), but keep the ratio of Transfection 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 plasmid DNA concentration, as described above and in the optimization section (pages 16–17), is recommended for every new plasmid and/or new cell line used.
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.

Observation	Possible Cause	Comments and Suggestions
	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: Composition of Buffers

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 7.4	10 mM Tris·Cl, pH 7.4 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 24–96 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 (2). 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” (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 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 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. 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 like 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 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 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 — based on activated dendrimer technology, and Effectene Transfection Reagent —representing a completely new class of lipid-based transfection reagent, have been designed to offer very high transfection efficiencies and reproducibility, while minimizing cytotoxic effects.

Plasmid DNA quality

The quality of plasmid DNA strongly influences the results of transfection experiments. Therefore only plasmid DNA of the highest quality, which is completely free of contaminating RNA, genomic DNA or proteins, should be used. DNA purified with QIAGEN and QIAfilter Plasmid Kits is ideally suited for transfection of most cell lines. For transfection of endotoxin-sensitive cells, we recommend using DNA purified with EndoFree Plasmid Kits. These kits efficiently remove bacterial lipopolysaccharide molecules 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) (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.

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Product Use Limitations

SuperFect Transfection Reagent and Effectene Transfection Reagent are developed, designed, and sold for research purposes only. They are 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.

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