For life science research only. Not for use in diagnostic procedures.

FuGENE 6 Transfection Reagent

Multi-component formulation for the transfection of eukaryotic cells

Cat. No. 1 815 091		0.4 ml	(80–120 transfections)
Cat. No. 1 814 443		1 ml	(200–300 transfections)
Cat. No. 1 815 075	Multi-pack	5 x 1 ml	(1000–1500 transfections)
Cat. No. 1 988 484	Custom pack	Inquire	(10 ml or 50 ml glass vials)

Store FuGENE 6 Transfection Reagent at –15 to –25ºC.

Instruction Manual

Version 5, September 2000

Roche

1. Preface

1.1 Table of contents

2. Product characteristics

Formulation

FuGENE 6 Transfection Reagent is a proprietary blend of lipids and other components supplied in 80% ethanol, sterile-filtered, and packaged in polypropylene tubes. Warm FuGENE 6 Transfection Reagent to ambient temperature (approximately 10 to 15 minutes at room temperature) prior to use. Always mix FuGENE 6 Reagent prior to use (vortex or inversion). After long periods of storage, FuGENE 6 Transfection Reagent may be slightly turbid due to the precipitation of some components. In most systems, the turbidity has no effect on the biological activity of the product.

***NOTE:* The new Custom-pack of FuGENE 6 Transfection Reagent is supplied in glass vials.**

Storage and stability

FuGENE 6 Transfection Reagent is stabilized for shipping at room temperature and for extended storage at –20°C through the expiration date printed on the label (two years from date of manufacture).

FuGENE 6 Reagent is shipped to you at room temperature.

Special handling

Do not aliquot FuGENE 6 Reagent from the original polypropylene tubes. Chemical residues in new plastic vials can significantly decrease the biological activity of the reagent.

***NOTE:* Evaluations have shown that FuGENE 6 Transfection Reagent remains fully functional even when vials are repeatedly opened (at least 6 times over a 3 month period), as long as the vials are recapped tightly and stored at –20°C between use. A 20% loss of ethanol due to evaporation does not substantially affect biological activity in COS-1 cells.**

3. Introduction

3.1 Product overview

Application

FuGENE 6 Transfection Reagent efficiently transfects a wide variety of mammalian cells and other cell types with high efficiency. Visit the FuGENE 6 Reagent web page for a current list of >250 successfully transfected cells lines: http://biochem. roche.com/techserv/fugene.htm

Number of tests

One milliliter of FuGENE 6 Reagent transfects approximately 200–300, 35 mm tissue culture dishes (total media volume of 2 ml each) with one of the following cell lines (HeLa, NIH 3T3, COS-1, COS-7, CHO-K1).

***NOTE:* The levels of expression of the gene of interest will vary with the cell line.**

continued on next page

3.1 Product overview, continued

Quality control *Functional analysis*	Three microliters of FuGENE 6 Transfection Reagent is combined with 1–2 µg of a reporter gene vector DNA, and used to transfect COS-1 cells in a monolayer (50–80% confluent) in the presence of 10% fetal bovine serum (FBS). Following transfection, the percentage of cells transfected is analyzed, and typically, 50%–70% of COS-1 cells express reporter gene protein.
Cytotoxicity analysis	COS-1 cells continuously exposed for 26 hours to FuGENE 6 Reagent, with or without DNA, in the presence of serum, and without a change of medium, are >90% viable by flow cytometric analysis based on propidium iodide-staining.

3.2 Background information

General transfection methods	Transfection is the general process of bringing foreign DNA into cells and monitoring protein expression. DNA transfection is essential for the study of gene function and gene regulation. • Common transfection techniques include calcium phosphate coprecipitation (1), electroporation (2,3), and the use of viral vectors (4). These methods have produced variable results in a variety of cell types (5–9). • Cationic liposome-mediated transfection methods (lipofection, cytofection) were an important addition to the previous methods (10). Additional classes of compounds known to mediate transfection include lipopolyamines (11) and dendrimers (12).
FuGENE 6 Transfection Reagent	FuGENE 6 Transfection Reagent is a multi-component lipid-based transfection reagent that complexes with and transports DNA into the cell during transfection. Benefits of FuGENE 6 Reagent include: • Provides very high transfection efficiency in many common cell types. • Demonstrates virtually no cytotoxicity even in many primary cell types. • Functions exceptionally well in the presence or absence of serum. • Requires minimal optimization.

4. Procedures and required materials

4.1 Before you begin

FuGENE 6 Reagent handling

NOTE: FuGENE 6 Transfection Reagent is unique as compared to other liposomal transfection reagents, and requires special handling. Review the following steps before proceeding further.

	Special handling steps
1	**Always** dilute FuGENE 6 Reagent by pipetting directly into serum-free medium. Do not allow the undiluted reagent to come into contact with any plastic surface. Refer to section 4.3.2, Preparation of FuGENE 6 Reagent:DNA complex. 
2	**Always** use more FuGENE 6 Reagent (µl volume) than DNA (µg mass). For details, see section 4.2.
3	**Always** store in the original polypropylene tubes. Do not aliquot. Chemical residues in new plastic vials can significantly decrease the biological activity of the reagent.
4	FuGENE 6 Reagent is very robust, and unlike other reagents, transfects most cell types equally well in the presence or absence of serum, reducing the number of handling steps.
5	**For most cell types:** All additional handling steps after the addition of the FuGENE 6 Reagent:DNA complex to the cells can be eliminated. Since FuGENE 6 Reagent is virtually nontoxic and transfects in the presence of serum, the transfection complex can be added to cells in serum-containing growth medium without washing (section 4.2). Additionally, the complex can be left on the cells until the time of the protein expression assay.

4.2 Factors for successful transfection

Overview

The following factors affect transfection reactions:

- FuGENE 6 Reagent working concentration
- Concentration and purity of nucleic acids
- Cell culture conditions
- Transfection in serum-containing or serum-free media
- Other media additives
- Verification of vector function
- High protein expression levels

continued on next page

4.2 Factors for successful transfection, continued

FuGENE 6 Reagent working concentration

For an initial experiment in a 35 mm culture dish, containing a monolayer of cells that is 50–80% confluent, transfect cells using FuGENE 6 Transfection Reagent (µl) to DNA (µg) amounts of 3:2, 3:1, and 6:1, respectively (refer to section 4.3.2 for important details of FuGENE 6:DNA complex preparation). For most cell types, these FuGENE 6 Reagent:DNA amounts provide excellent levels of transfection.

NOTE: Subsequent optimization may further increase efficiency in your particular application. Hold the amount of DNA constant while increasing the volume of FuGENE 6 Reagent (as illustrated in Figure 2). Transfection efficiency is greatly reduced when the amount of DNA exceeds a ratio of 3:2 (volume FuGENE [µl]): mass DNA [µg], see Figures 1 and 2).

Concentration and purity of nucleic acids

It is critical to accurately determine the concentration of your DNA using 260 nm absorbance. For initial experiments, avoid possible cytotoxic effects by using highly purified nucleic acids (*e.g.*, cesium chloride gradient or column purified), free of traces of residual cesium chloride. Once transfection conditions are established, plasmid preparation with higher endotoxin levels can be tested. ***NOTE: Use a plasmid concentration between 0.02 and 2.0 µg/µl.***

Cell culture conditions

Use cells that are healthy, proliferating well, and plated at a consistent density to minimize both intra- and interexperimental variance in transfection efficiency.

NOTE: Use only regularly passaged cells in a log-growth phase.

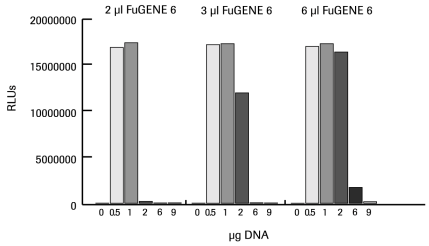

Figure 1. Effect of DNA amount on transfection efficiency using FuGENE 6 Transfection Reagent. COS-1 cells (1.3 x 10^5) were plated in 35 mm dishes 18 hours prior to transfection. Cells were transfected with one of three fixed volumes of FuGENE 6 Reagent and various quantities of pCMV-SEAP plasmid. Expression of the SEAP reporter gene was determined with the chemiluminescent SEAP Reporter Gene Assay (Cat. No. 1 779 842).

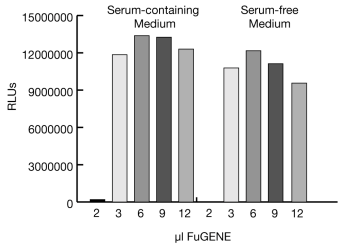

Figure 2. Effect of the presence or absence of serum on transfection efficiency with FuGENE 6 Reagent. COS-1 cells (1.3 x 10^5) were plated in 35 mm dishes. The next day, different volumes of FuGENE 6 Reagent were used to transfect cells with 2 µg pCMV-bGal in the presence or absence of serum. Cells were lysed using 0.25 ml of lysis buffer, and β-galactosidase expression was determined by analyzing 5 µl cell lysate with the chemiluminescent β-Gal Reporter Gene Assay (Cat. No. 1 758 241).

Transfection in serum-containing or serum-free media

FuGENE 6 Reagent produces highly efficient transfection both in the presence and absence of serum (Figure 2). In some cell types, transfection efficiency may improve in the presence of serum, and in other cell types, higher transfection efficiency may be achieved in reduced-serum medium, or under serum-free conditions. The same type of cell culture medium routinely used for culturing cells can also be used during transfection.

If low or reduced serum conditions are used for transfection, return cells to normal serum concentrations by adding additional serum or changing the medium 3–8 hours post transfection.

NOTE: ***Prepare the FuGENE 6 Reagent:DNA complex in medium that does not contain serum.***

Other media additives

In some cell types, antimicrobial agents (*e.g.*, antibiotics and fungicides) commonly included in cell culture media, have been observed to adversely affect the transfection efficiency of FuGENE 6 Transfection Reagent. Exclude additives for initial experiments if possible. Once high-efficiency conditions have been established, these components can be added, however, you will need to monitor your transfection results.

Verification of vector function

Prior to transfecting cells with a new vector construct, verify and (if necessary) optimize transfection conditions with a known positive control reporter gene construct.

- Use the same vector backbone as your construct, where a reporter gene was previously inserted.
- Determine the transfection efficiency by measuring the level of reporter protein expression with a reporter gene assay (CAT, β-Gal, luciferase, SEAP, or hGH, see section 5.4, Related products).
- Confirm insert size by performing PCR with the Intelli-Search Bacterial Colony Screen Kits (section 5.4, Related products).
- Sequence across the flanking vector insert regions to verify the integrity of your new construct.

High protein expression levels

High levels of expression of certain proteins (*e.g.*, GFP) may be cytotoxic for some cell types. Refer to section 5.1, Troubleshooting guide.

4.3 Standard protocol for transient and stable transfection

Additional required reagents

- Sterile, serum-free culture medium, (optional: add 12.5 mM HEPES buffer to serum-free medium).
- DNA stock solution in 1x sterile TE buffer or sterile water with a concentration determined by 260 nm absorption.

4.3.1 Preparation of cells for transfection

Adherent cells

Plate the cells one-day before the transfection experiment. The appropriate plating density will depend on the growth rate and the condition of the cells. Use cells that are 50–80% confluent on the day of the experiment. Plating most cell lines at 1–3 x 10^5 cells in 2 ml in a 35 mm culture dish (or 6-well plate) will achieve this density after overnight incubation.

***NOTE:* Adjust the number of cells accordingly if using culture plates of different sizes (see Table 1 below).**

Suspension cells

Use freshly passaged cells at a concentration between 5 x 10^4/ml to 1 x 10^6/ml (2 ml in a 35 mm culture dish or 6-well plate). Determine cell number based on your needs and cell type to be transfected.

***NOTE:* It is usually not necessary to wash the cells prior to the addition of the transfection reagent:DNA complex because performance is independent of the presence or absence of fetal bovine serum in the cell culture medium (Figure 2).**

Table 1

Refer to the following table when setting up your transfection reactions. This table is based on a transfection reagent:DNA ratio of 3:1. Refer to section 4.3.2 to use other ratios.

Type of dish or plate	Surface area per well or plate (cm^2)	Total media volume per well or plate (ml)	Starting volume of FuGENE 6 Reagent (µl/ well or plate)	Starting mass of DNA (µg/ well or plate)
60 mm	21	4	6.0	2.0
35 mm	8	2	3.0	1.0
6-well	9.4	2	3.0	1.0
12-well	3.8	1	1.5	0.5
24-well	1.9	0.5	0.6	0.2
96-well	0.3	0.1	0.15	0.05

4.3.2 Preparation of FuGENE 6 Reagent:DNA complex

Adherent and suspension cells in a 35 mm culture dish

The following ratios have been optimized for adherent cells. These ratios also work with suspension cells.

Use FuGENE 6 Reagent:DNA amounts of 3:2, 3:1, and 6:1 (µl and µg, respectively) in each well of a 6-well plate or 35 mm culture dish (described below).

FuGENE 6 Reagent:DNA ratio	FuGENE 6 Reagent volume (µl)	DNA volume (µg)
3:2	3	2
3:1	3	1
6:1	6	1

Step	Preparation of complex
1	In a small sterile tube, add sufficient serum-free medium as diluent for FuGENE 6 Transfection Reagent, to a total volume of 100 µl. Add 3 to 6 µl of FuGENE 6 Reagent directly into this medium. The order of addition is critical. The serum-free medium must be pipetted into the tube first. Tap gently to mix. ***NOTE: To avoid adversely affecting transfection efficiency, do not allow undiluted FuGENE 6 Reagent to come in contact with plastic surfaces other than the pipette tip.***
2	Add 1–2 µg DNA solution (0.02–2.0 µg/µl) to the prediluted FuGENE 6 Reagent from Step 1. Use a total volume of DNA solution between 0.5–50 µl.
3	Gently tap the tube to mix the contents. DO NOT VORTEX. Incubate for a minimum of 15 minutes at room temperature. Continued incubation for up to 45 minutes (for some cell lines up to 2 hours) will not affect the transfection efficiency in most cell types. Go to section 4.3.4, Transfection of cells.

NOTE: To further optimize conditions, test a broader range (2–15 µl) of FuGENE 6 Transfection Reagent, while keeping the amount of DNA constant at 1–2 µg. Refer to section 5.1, Troubleshooting.

4.3.3 Scale-up for large transfection experiments

Preparation of master mix complex

To prepare transfection complexes using the same DNA for many parallel experiments, proportionally increase the quantity of all components, including the serum-free medium, FuGENE 6 Transfection Reagent, and the vector DNA.

4.3.4 Transfection of cells

Transfection procedure

The following procedure has been optimized for adherent cells (Step 1). Suspension cells can be transiently transfected using the same procedure after transfer to a new plate or flask.

Step	Transfection procedure
1	Dropwise, add the complex mixture from Step 3 (section 4.3.2) to your cells, distributing it around the well. Swirl the wells or flasks to ensure even dispersal.
2	Return the cells to the incubator until the time of the reporter gene assay. ***NOTE: There is no need to remove the reagent:DNA complex from the cells prior to the reporter gene assay. In our experience, exposure of most common laboratory cell types (COS-1, CHO-K1, HEK-293, HeLa) to the reagent: DNA complex until the time of the reporter gene assay (24–48 hours later), has produced no adverse effects, however, this may need to be determined for your particular cell type.*** If you observe cytotoxicity with the FuGENE 6:DNA complex, refer to section 5.1, Troubleshooting. For stable transfection experiments, the complex containing medium can be left unchanged until the cells need to be fed.
3 (optional)	Use serum-free medium during the transfection procedure, and replace the medium with serum-containing medium 3–8 hours after transfection, or add serum directly to wells.

4.3.5 Cotransfection experiments

Suggestions

Cotransfection experiments with FuGENE 6 Reagent have been performed simultaneously using up to seven different plasmids. Increase the amount of transfection reagent in proportion to the amount of total µg DNA when performing cotransfection experiments.

NOTE: Always use excess volume of FuGENE 6 Reagent over the total final mass of DNA (section 4.2).

4.3.6 Optimization of transfection efficiency and protein expression levels

Optimization factors

Consider the following factors when optimizing your transfection reaction.

- FuGENE 6 Reagent:DNA ratio
- Cell density and growth phase
- Cell passage history
- Number of hours to measurement of reporter gene activity

4.3.7 Measurement of protein expression

Variables

Incubate the cells 4–72 hours. The length of incubation depends upon the transfected vector construct, the cell type being transfected, and the protein being expressed. After this incubation period, measure protein expression using an assay appropriate for your system.

4.4 Large-scale transfection using FuGENE 6 Reagent for transient protein expression

Overview

- FuGENE 6 Reagent has been successfully used to greatly increase the speed of drug discovery in pharmaceutical drug screening programs. This is accomplished by eliminating the need to establish stable cell lines expressing receptors or ligands of interest.
- Large-scale transient transfection experiments using FuGENE 6 Reagent yield cells expressing molecules of interest at high levels. The exceptionally low toxicity of the reagent is especially beneficial. After transfection, cells retain most normal physiological functions, and can therefore serve as targets for various screening applications. In addition, several cumbersome steps required with first generation transfection reagents can be eliminated with the use of FuGENE 6 Reagent (see Table 2). As a result, you can easily screen thousands of small molecules within a very short time period using transiently transfected cells.
- FuGENE 6 Reagent has also been used to express pharmacologically interesting receptors at high density in cells, followed by the preparation of membrane fractions for classical drug binding studies (unpublished data). In more complex studies, cells can be transfected with molecules of interest, and then the whole cells can be used in screening assays while monitoring physiological activity with specific indicators.

Table 2.

Step	FuGENE 6 Transfection Reagent	First generation transfection reagents
Count and plate cells in FBS medium	÷	÷
Incubate overnight		÷
Remove FBS-containing media		÷
Replace media without FBS		÷
Add transfection reagent:DNA complex	÷	÷
Remove transfection reagent:DNA complex after 4–6 h		÷
Replace with fresh serum-containing media		÷
Total transfection steps	2	7

4.4.1 Optimization in high through-put screening (HTS) applications and protein production

Optimization of large-scale transfections

Refer to the following table for a list of factors that can shorten the transfection procedure, and help attain maximal levels of protein expression.

Optimization of FuGENE 6 Reagent/DNA ratio	• In some systems, increasing the amount of both FuGENE 6 Reagent and DNA (more than ten fold higher than the recommended amounts), can continue to increase the level of protein expression. • The very low cytotoxicity of FuGENE 6 Reagent permits both the FuGENE 6 Reagent and DNA levels to be tested at these high levels without adversely affecting cell viability.
Transfection of cells immediately following trypsinization	• Use FuGENE 6 Reagent to transfect some cell lines immediately following trypsinization and just prior to or after plating. • This will substantially reduce set-up time by eliminating the need to wait 24 hours before transfection.
Transfection of adherent cells adapted for suspension growth	• In some cases, adherent cells may be adapted for suspension growth, reducing requirements for expensive sterile plastic tissue culture vessels, and enable the production of transiently transfected cells on a very large scale. • HEK-293 cells have been transfected while in suspension growth using FuGENE 6 Reagent (unpublished data).
Effect of media and media components, including sera	• Different media and media components may influence the level of transfection efficiency and the subsequent growth of the transfected cells, as well as expression of the recombinant protein. • Test different media and optimize the level of each medium component for these effects. Although it is not usually necessary to remove the transfection reagent:DNA complex following the transfection step, it is necessary to feed your cells with fresh media for extended growth periods. This is especially important if the transfected cells are allowed to continue to grow for 3–7 days, allowing for maximal protein expression.

5. Appendix

5.1 Troubleshooting table

Low transfection efficiency

Refer to the following table if you observe a low transfection efficiency.

Problem	Possible cause	Recommendation
Low transfection efficiency	Nucleic acids of poor quality or insufficient quantity	Verify the amount or quality of nucleic acid: • Use only high-quality plasmid preparations, see section 4.2. • Use DNA at a concentration of 0.02–2.0 µg/µl. • Verify that the transfected plasmid construct contains appropriate promoters and other sequences required for protein expression in the cell line being transfected. • Perform a control transfection experiment with a commercially available transfection-grade plasmid preparation (*e.g.*, the β-gal control vectors supplied with the Mammalian Expression Vectors for Epitope Tagging (Cat. No. 1 814 664). ***NOTE:* Endotoxins are reported to be cytotoxic to some very sensitive cell lines (e.g., Huh-7) and primary cultures (13). When using FuGENE 6 Reagent for many common cell types, it may be possible to use DNA containing higher endotoxin levels.**
	Insufficient number of cells were used	Use adherent cells at 50–80% confluency.
	FuGENE 6 Reagent was aliquoted and stored in a new container	Check that FuGENE 6 Reagent is stored in the original container.
	FuGENE 6 Reagent came into contact with plastic	Repeat transfection, carefully pipetting FuGENE 6 Reagent directly into the serum-free medium.
	Complex was formed in serum-containing medium	Check original bottle of medium used for complex formation. Repeat experiment using new bottle of medium that does not contain any additives (*e.g.*, serum, antibiotics, growth enhancers, etc.)

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5.1 Troubleshooting table, continued

<table>
<tr><th>Problem</th><th>Possible cause</th><th>Recommendation</th></tr>
<tr><td>Low transfection efficiency</td><td>A suboptimal FuGENE 6:DNA ratio was used</td><td>
• Optimize the FuGENE 6 Reagent:DNA ratio according to the following procedure. <u>Note:</u> Always use more FuGENE 6 Reagent (µl) than DNA (µg). For example, combine 3 µl FuGENE 6 Reagent with 1–2 µg DNA for a 35 mm culture dish (6-well plate).
• Prepare FuGENE 6:DNA mixtures according to the following table. Do not allow FuGENE 6 Transfection Reagent to come in contact with the plastic tube before dilution with serum-free medium.
<table>
<tr><td>Label six tubes</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td></tr>
<tr><td>Add serum-free media (µl)</td><td>97</td><td>97</td><td>97</td><td>94</td><td>94</td><td>94</td></tr>
<tr><td>Add FuGENE 6 Reagent (µl)</td><td>3</td><td>3</td><td>3</td><td>6</td><td>6</td><td>6</td></tr>
<tr><td>Add DNA (µg)</td><td>0.5</td><td>1</td><td>2</td><td>1</td><td>2</td><td>3</td></tr>
</table>
1 Tap the tubes gently. Mix thoroughly, but do not vortex.
2 Incubate at room temperature for 15-45 minutes.
3 Add each FuGENE 6 Reagent:DNA mixture to a 35 mm culture dish or one-well of a 6-well plate. Swirl the plates.
4 If you raise the DNA concentration (e.g., in a cotransfection experiment), proportionally increase the amount of FuGENE 6 Transfection Reagent.
• If your cell line is not easily transfected by the above FuGENE 6 Reagent:DNA ratios, test a wider range of ratios, including 2–15 µl FuGENE 6 Transfection Reagent per 1–2 µg DNA, per 35 mm culture dish.
• If no transfection is observed, repeat the experiments with DOTAP or DOSPER Liposomal Transfection Reagent (see section 5.4, Related products).
</td></tr>
</table>

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5.1 Troubleshooting table, continued

Signs of cytotoxicity

Refer to the following table if you observe signs of cytotoxicity.

Problem	Possible cause	Recommendation
Signs of cytotoxicity ***NOTE: FuGENE 6 Transfection Reagent has proven to be virtually non-toxic to most cell types.***	Selection antibiotic added too soon	Repeat transfection and wait an additional 24 to 48 h before adding the selection antibiotic, to allow for sufficient protein production.
	Selection antibiotic at too high a concentration	Repeat transfection using several lower concentrations of selection antibiotic.
	Transfected protein is cytotoxic or is produced at high levels	Reduced viability or slow growth rates may be the result of high levels of protein expression, as the cells' metabolic resources are directed toward production of the heterologous protein. The expressed protein may also be toxic to the cell at the level expressed. To analyze cytotoxicity, prepare experimental controls as described. Prepare extra wells containing: a. Cells that are not transfected. b. Cells transfected with DNA alone (*e.g.*, without FuGENE 6 Transfection Reagent) c. Cells treated with FuGENE 6 Reagent alone (no DNA added). • Compare transfected cells with the experimental construct, to the wells containing these experimental controls. • Consider repeating the experiment with a secreted reporter gene assay such as SEAP, hGH, or a standard β-gal control vector (see low transfection efficiency above). Cells secreting SEAP should show little to no evidence of cytotoxicity.
	The culture may be contaminated with mycoplasma	• Use the Mycoplasma Detection Kit or Mycoplasma PCR ELISA (see section 5.4) to determine if the culture is contaminated. • Treat the cells with BM Cyclin to eliminate the mycoplasma. Alternatively, start the transfections over with a fresh uncontaminated culture.
	Cells may not be healthy (*e.g.*, malfunctioning incubator, media problems)	Assess physiological state of cells and the incubation conditions (*e.g.*, CO_2 and temperature levels). Perform the same controls as suggested above (for cytotoxicity), to eliminate influence of transfection reagent or nucleic acid.

continued on next page

5.1 Troubleshooting table, continued

Problem	Possible cause	Recommendation
Signs of cytotoxicity (continued)	Plasmid preparation contaminated with large amounts of endotoxin	Endotoxin is reported to be cytotoxic to some very sensitive cell lines (*e.g.*, Huh-7) and primary cultures (13). By using FuGENE 6 Reagent for many common cell types it may be possible to use DNA containing higher endotoxin levels. See section 4.2.
	If above tests prove negative, FuGENE 6 Reagent may be cytotoxic to your specific cell type	If you are using a very sensitive cell line, some steps can be taken to minimize cytotoxicity: • Perform the transfection in the presence of FBS. • Reduce the time of exposure to the transfection reagent:DNA complex, 2–3 h maximum, then replace the medium. • Perform the transfection at a higher cell density. • Use different ratios of FuGENE 6 Reagent:DNA

NOTE: Refer to the FuGENE 6 web page for additional help: ***http://biochem.roche.com/techserv/fugene.htm***

5.2 How to contact Roche Molecular Biochemicals

Three ways to contact us

To contact Roche Molecular Biochemicals for technical assistance, choose one of the following:

If you are using…	THEN…
the Internet	access our web site at http://biochem.roche.com
E-mail	Identify the address that corresponds to your particular location on the back cover of this instruction manual.
the telephone	Identify the address that corresponds to your particular location on the back cover of this instruction manual.

5.3 References

General transfection techniques

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Endotoxin levels in primary cultures

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5.4 Related products

Transfection Reagents	Cat. No.	Pack Size
DOSPER Liposomal Transfection Reagent	1 781 995 1 811 169	2 ml (5 x 0.4 ml) 0.4 ml
DOTAP Liposomal Transfection Reagent	1 202 375 1 811 177	2 ml (5 x 0.4 ml) 0.4 ml

Selection Antibiotics	Cat. No.	Pack Size
Geneticin (G 418)	1 464 973 1 464 981 1 464 990	250 mg 1 g 5 g
Hygromycin B	843 555	1 g

Reporter Gene Assays	Cat. No.	Pack Size
β-Gal ELISA	1 539 426	1 kit (192 tests)
β-Gal Reporter Gene Assay, chemiluminescent	1 758 241	1 kit (500 assays, MTP format; 250 assays, tube format)
β-Gal Staining Set	1 828 673	1 set (100 tests)
CAT ELISA	1 363 727	1 kit (192 tests)
CAT Staining Set	1 836 358	1 set (100 tests with 3.5 cm dishes)
Complete Protease Inhibitor Cocktail Tablets	1 697 498	20 tablets
hGH ELISA	1 585 878	1 kit (192 tests)
Luciferase Reporter Gene Assay, high light intensity	1 669 893 1 814 036	200 assays 1000 assays
Luciferase Reporter Gene Assay, constant light signal	1 897 667	1000 assays
SEAP Reporter Gene Assay, chemiluminescent	1 779 842	1 kit (500 assays, MTP format; 250 assays, tube format)
X-Gal	651 745 651 737 100 081 745 740	250 mg 25 mg 100 mg 1 g

Mycoplasma Detection and Elimination	Cat. No.	Pack Size
BM Cyclin	799 050	37.5 mg (for 2 x 2.5 l medium)
DAPI (fluorescent detection of mycoplasma)	236 276	10 mg
Mycoplasma Detection Kit	1 296 744	1 kit (25 tests)
Mycoplasma PCR ELISA	1 663 925	1 kit (96 reactions)

continued on next page

5.4 Related products, continued

Vectors and Screening Kits	Cat. No.	Pack Size
pXB Bacterial Expression Vector (N-terminal)	1 814 591	20 µg
pBX Bacterial Expression Vector (C-terminal)	1 814 621	20 µg
pXM Mammalian Expression Vector (N-terminal)	1 814 699	20 µg
pMX Mammalian Expression Vector (C-terminal)	1 814 737	20 µg
pHB6 Bacterial Expression Vector (N-HA+His_6-C)	1 814 575	20 µg
pVB6 Bacterial Expression Vector (N-VSV-G+His_6-C)	1 814 583	20 µg
pBH Bacterial Expression Vector (HA-C)	1 814 605	20 µg
pBV Bacterial Expression Vector (N-VSV-G-C)	1 814 613	20 µg
pVM6 Mammalian Expression Vector (N-VSV G+His_6-C)	1 814 672	20 µg
pHM6 Expression Vector (N-terminal HA tag/ C-terminal HIS-6 tag, and β-Gal control vector)	1 814 664	20 mg
pMH Mammalian Expression Vector (HA -C)	1 814 702	20 µg
pMV Mammalian Expression Vector (VSV-G-C)	1 814 729	20 µg
Intelli-Search B Bacterial Colony Screen (for identification of inserts in bacterial expression vectors)	1 814 842	100 reactions
Intelli-Search M Bacterial Colony Screen (for identification of inserts in mammalian expression vectors)	1 814 834	100 reactions

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