



FuGENE[®] HD Transfection Reagent

Application Note No. 1/September 2006



Tips and tricks on how to improve transfection experiments

Successful Transfection is based on Choosing the Right Reagent and Using it Appropriately

Introduction

Under normal conditions, mammalian cells take up and express externally applied DNA with very low efficiency. This is mostly due to the lipid bilayer of the eukaryotic cell membrane, which poses a significant barrier to the entry of charged molecules into a cell. Several transfection methods have been developed to overcome this problem. With these methods, the study of gene expression in cultured cells using DNA or RNA transfection has become routine.

Briefly, transfection is the delivery of DNA, RNA, proteins, and macromolecules into eukaryotic cells. Goals for transfection include the study of gene regulation as well as protein expression and function. There exist several different well-established methods for the delivery of molecules, particularly nucleic acids, into eukaryotic cells. These transfection methods are based on three different strategies. (For details, see www.roche-applied-science.com/transfection)

There Is No Universal Transfection Reagent

Not all transfection methods can be applied to all types of cells or experiments. The different methods vary greatly with respect to the level of gene expression that can be achieved.

Moreover, no single technique is suitable for the multitude of different cellular systems used for transfection experiments. Each transfer method may have advantages or disadvantages, depending on, for example, the cell type to be transfected (especially true for difficult cell lines), the molecule to be transfected (DNA, RNA, oligonucleotides, proteins), or even the demands of high-throughput applications. In all cases, however, the success of transfection

depends on transfection efficiency, low cytotoxicity, and reproducibility. To ensure a highly efficient transfection, you need to choose a reliable transfection technology and the reagents that work optimally under your cell culture conditions.

To select the transfection reagent that best meets your needs, check existing references about your special cell type or application, study the characteristics of the various reagents (available on the internet; see www.roche-applied-science.com/transfection) and, if possible, test several different reagents using the protocols recommended for that reagent.

Considerations for Specific Applications

Cell Physiology

To study cell physiology, or use cells for target validation, it is important to be sure that the transfection process itself does not alter the pathway of interest, or if it does, does so minimally. If you transfect with reagents that are deleterious to the cells, it can be difficult to differentiate between the effects of transfection itself and the effect of the specific gene being transfected. For this reason, it is critical to always transfect with empty vectors and vectors containing irrelevant genes. Only then can you begin to evaluate the effects of the gene of interest.

When performing transfections, one should strive to maximize transfection efficiency and minimize morphologic changes in the cells visible by phase contrast microscopy. We have done studies using two of our

reagents, FuGENE® 6 and FuGENE® HD Transfection Reagents in which we have transfected cells and, using microarray analysis, determined the number of genes that were up or down regulated. These reagents had many fewer off-target effects on test cells compared to a well known competitive reagent (see references).

Protein Production using Transient Transfection

Previously, to produce high levels of protein, you had to transfect cells, then select stable cell lines for expansion and accumulation of protein. Selection of such cell lines often took weeks or months, due either to the cytotoxicity of the reagent or inefficient transfection. If the transfection reagent was cytotoxic, then fewer cells survived and the protein yield was consequently low. If only a few cells were transfected, then untransfected cells rapidly outgrew the transfected cells, and little protein was produced.



Now, to produce high levels of protein, you have a better option, *i.e.* using a gentle reagent that transfects most of the cells. With FuGENE® HD Transfection Reagent, you can produce high yields of protein 3-7 days after transfection because it is relatively non-cytotoxic, transfects most of the cells, and results in high yield per cell.

However, some proteins are produced at higher levels than other proteins. Thus, critical variables for high levels of protein expression include:

- **Cell line (some cell types are higher producers than others)**
- **Plasmid back bone (*e.g.*, enhancers, promoters, transcriptional regulatory elements)**
- **Protein produced (some proteins simply are not well produced)**
- **cDNA sequence of protein (*e.g.*, codon optimization)**
- **Medium (nutrition, wastes, inhibitors of transfection)**

When these factors are optimized and the optimal ratio and amount of complex (Reagent-Plasmid) are added, levels comparable to average levels of stable clones can be obtained.

Transient versus Stable Transfection

The first step in preparing a stable cell line is choosing a vector that contains both a selectable marker and the gene of interest. The selectable marker is usually one that produces a protein or enzyme that allows cells to grow in the presence of an otherwise toxic chemical.

Once the plasmid vector is chosen, the transfection is done the same way for either transient or stable expression. The transfected cells are allowed to grow, at least overnight in standard medium, before the selective agent is added. This gives the cells time to express sufficient quantities of the protein that allows them to overcome the selection agent. The cells are then grown in the presence of the selection agent. Cells that do not have the plasmid are killed; only transfected cells are able to grow. In some instances, the selection agent is continuously added to the culture medium to maintain selective pressure on the cells.

Current Transfection Reagents Provided by Roche Applied Science

Roche Applied Science offers a variety of reagents for different transfection methods. Below is an overview of the different reagents.

Multicomponent reagents

Novel multicomponent reagents combine high transfection efficiency with low cytotoxicity, and eliminate the need for extensive optimization. Roche Applied Science offers several multicomponent reagents:

■ **FuGENE® 6 Transfection Reagent** (Cat. Nos. 11814443001, 11815091001, 11988387001, and 11988484001)

FuGENE® 6 Transfection Reagent is an optimized blend of lipids and other proprietary compounds. This reagent combines high transfection efficiency with exceptionally low cytotoxicity. In contrast to cationic liposome-based transfection reagents, it can produce successful transfections the first time it is used (without requiring optimization of transfection conditions). The reagent has been used to successfully transfect more than 700 cell types, including many primary cells.



Since it is very easy to use, and gentle enough to be used on freshly trypsinized cells, it is ideal for a variety of high-throughput screening applications.

■ **FuGENE® HD Transfection Reagent.** (Cat. Nos 04709691001, 04709705001, 04709713001)

FuGENE® HD Transfection Reagent is a “next-generation” transfection reagent, which is free of animal- or human-derived components, stable at room temperature, and sterile (0.1µm) filtered. FuGENE® HD Transfection Reagent is a multipurpose reagent, suitable for diverse applications. For example, it:

- Has an easily optimizable protocol that allows it to transfect a wide range of eukaryotic cells, including animal and insect cells, with minimal cytotoxicity.
- Offers excellent transfection efficiency for many cell lines that are not transfected well by other reagents, such as MCF-7, RAW 264.7, PC-3, HeLa, MA-10, HepG2, SH-SY5Y, A7r5, STO, SCC-61, STSAR-90, SQ20B, T98G, A375, A549 and stem cells.
- Is particularly effective in protein expression experiments over extended periods, since it can transfect many adherent and suspension-adapted cell lines commonly used for protein expression (*e.g.*, CHO-K1, HEK 293, and insect cells).
- Allows cells to be grown either in serum-containing or serum-free medium, yet still produce high levels of recombinant proteins.

FuGENE® HD Transfection Reagent even allows high levels of expression in high levels (up to 100%) of serum. Thus, it is the reagent of choice for studies under these conditions.

■ **X-tremeGENE siRNA Transfection Reagent**
(Cat Nos. 04476093001, 04476115001)

■ **RNA interference (RNAi)** is a powerful method for inhibiting the expression of a particular gene. RNAi experiments require a transfection reagent that is specially optimized for siRNA delivery into mammalian cells. Such a reagent is X-tremeGENE siRNA Transfection Reagent, which forms a complex with siRNA and effectively delivers it into eukaryotic cells. The reagent

transfects many commonly used cell types very efficiently, including HeLa, NIH 3T3, HEK-293, CHO-K1, COS-7, and several “hard-to-transfect” cell lines. In co-transfection-based RNAi experiments, X-tremeGENE siRNA Transfection Reagent supports both high protein expression and effective gene knockdown.

Note: This reagent has low cytotoxicity. It also functions exceptionally well in the presence or absence of serum; it does not require a media change, either before transfection or after addition of transfection complex.

Cationic liposomal reagents

If a cationic lipid is mixed with a neutral lipid, unilamellar liposome vesicles are formed that carry a net positive charge due to the highly positive amine groups on the cationic molecules. Nucleic acids can adsorb to these vesicles and gain access to the inside of cells, most likely by fusion of the liposome with the plasma membrane to form an endocytic vesicle. Under carefully optimized conditions, liposome-mediated transfection methods are highly efficient and are much easier to use than earlier methods. Roche Applied Science offers three liposome-based reagents for transfection of DNA, RNA, and oligonucleotides:

■ **DOTAP Liposomal Transfection Reagent**
(Cat. Nos. 11202375001, 11811177001)

DOTAP is a monocationic reagent.

■ **DOSPER Liposomal Transfection Reagent**
(Cat. Nos. 11811169001, 11781995001)

DOSPER is a polycationic reagent.

■ **X-tremeGENE Q2 Transfection Reagent**
(Cat. Nos. 03045595001, 03036421001)

X-tremeGENE Q2 Transfection Reagent was designed and optimized for transfection and high-level protein expression in K-562 and Jurkat cells.

Note: This proprietary reagent is supplied as a dried lipid film.

For a complete list of cell types successfully transfected with Roche Applied Science Transfection Reagents, see our *Transfection Database in the Internet* at www.roche-applied-science.com/transfection

Keys To Successful Transfection Experiments

In this section we explain how key components of the transfection system may influence the efficiency of a cell transfection experiment and, when appropriate, give tips on how to make them work in your favor.

Tissue Culture Reagents

General tips: Optimize the growth conditions of your cells. Use only fresh media and additives, and minimize variations in the reagents used.

Basic Medium – Various commercial media are currently used (e.g., RPMI 1640 and DMEM). Medium constituents include nutrients (amino acids, glucose), vitamins, inorganic salts, and buffer substances. Some constituents are quite unstable and thus may cause problems if they are not fresh when used. Always protect medium from light. Some components and buffers, such as HEPES, are broken down into cytotoxic components when exposed to light.



Phenol red offers protection from some of the effects of HEPES breakdown, but cytotoxicity could be a problem even in applications where phenol red free medium is used, e.g. luciferase assays.

Fetal Bovine Serum – Serum is an extremely complex solution of albumins, globulins, growth promoters, and growth inhibitors. The age, nutritional level, and health of the animals from which the serum is obtained affect the quantity and quality of these components. The serum is subject to significant biological variation.

Additives – Some cells depend on substances that are essential for viability or cell division (e.g., growth factors, trace elements, essential metabolites, and protein).

CO₂ incubator – Cells are grown at 37°C in a CO₂ incubator at 95% relative humidity. CO₂ is used to control the pH. Cell physiology is highly sensitive to pH variations, so most cell culture media contain a bicarbonate buffer. Some media require a concentration of 5% CO₂ for effective pH control, while other media require 10% CO₂. Check with the supplier of your medium for the appropriate concentration. Inconsistent conditions (temperature, humidity, and CO₂) in the incubator can cause plate-to-plate variability in the results. Pollution, chemicals, or fungal/bacterial contamination from the incubator may also affect cell physiology.

Cells

General tips: Keep an eye on your cells; ensure they are in good condition. Before starting transfection, develop a suitable plating protocol for optimal cell density from the beginning to the end of transfection.

Increase Success – Cells are a key component and can be the greatest variable affecting the consistency and quality of results. To help eliminate these concerns, Roche Applied Science has partnered with ATCC® (American Type Culture Collection). To ensure the quality of the cells to be transfected, Roche Applied Science recommends using freshly obtained cell lines from ATCC®.

Dividing versus Non-dividing Cells – Dividing cells tend to be more amenable to uptake and expression of foreign DNA than quiescent cells. Thus for most transfections, cells are plated the day of, or the day prior to transfection. It is also important that the cells are not overgrown at the time they are plated for transfection. Since FuGENE® 6 and HD Transfection Reagents are so gentle to the cells, it is possible to simultaneously plate and transfect adherent cells.

Furthermore, mitogenic stimuli (*e.g.*, virus transformation, growth factors, conditioned media, and feeder cells) are often used to activate primary cells.

Adherent versus Suspension Cells – Adherent and suspension cells differ significantly in transfection efficiency. Cells that by nature tend to be in suspension (*e.g.* HL 60, Jurkat) are very difficult to transfect. In contrast, cells that by nature are adherent (*e.g.* HEK, CHO) can be adapted to grow in suspension.



This is often done in serum-free medium containing special additives that inhibit transfection. With care, the cells can be adapted to grow in suspension without additives. If the suspension-adapted adherent cells are grown without these additives, they can be transfected.

The plasma membranes of the two types of cells (those naturally in suspension and those that are adapted to grow in suspension) are different. There is speculation that a limiting step in transfection is uptake of molecules (DNA or RNA complexed to transfection reagent) by endocytosis; however, a plausible mechanistic explanation at the molecular level does not yet exist. The differences in membrane structure among cells may be a partial cause of the inherent difficulty to transfect certain cell types. Therefore, the search for more efficient transfection reagents is mainly empirical, particularly for cells that naturally grow in suspension.

Splitting Protocol – Before splitting, adherent cells must be trypsinized in order to remove them from the substrate. This routine step causes a severe disruption of normal cellular functions. Therefore, differences in the splitting protocol (*e.g.*, extension of trypsinization, inactivation of trypsin, and time until transfection starts) could affect transfection experiments.

If cells are too confluent at the time of transfer, clumps instead of individual cells are plated. For some cell/reagent combinations, it is possible that the altered state of the plasma membrane may affect the optimal amounts and ratio of reagent and DNA.

Passage Number – The passage number indicates how often a cell line has been split (normally within one lab). In some cases, the exact passage number, since the line was established, is unknown. Some cell lines may be more unstable than others and may change over time in culture depending on the line and culture conditions. Different culture conditions could lead to clonal selection. Thus cell lines with the same name could significantly differ with respect to physiology and morphology (and transfectability).

Generally, cells are more difficult to transfect during the first passage or two after cryopreservation or until they have fully recovered. Transfection efficiency varies from cell line to cell line. Some lines exhibit constant transfection efficiencies over many passages while others show differences at much lower passages.

Cell Number (Grade of Confluency) – Cell lines divide exponentially when there is space on the substrate (tissue culture dish). For normal cells, the growth rate is inhibited by cell density (contact inhibition), however cancer cell lines will continue to grow and may pile up on each other. Depletion of nutrients and build-up of metabolic waste products affect all cells. Cells that have been stressed by lack of nutrients are not suitable for transfection.

Rates of reporter gene expression correlate with the cell number and cell health at the beginning of transfection and their subsequent growth prior to cell lysis.

Culture Contamination – Cultures can be contaminated with bacteria, yeast, fungi, viruses, *mycoplasma*, even other cell types. All types of contamination lead to erroneous results.

Mycoplasma contamination

Mycoplasma contamination, which is present in 5 – 35% of all cell cultures, can alter growth characteristics, enzyme patterns, cell membrane composition, chromosomal structure, and transfection efficiency. Specifically, *mycoplasma* interferes with transfection methods that use lipid, DEAE-dextran, calcium phosphate, or adenovirus mediation, resulting in low or atypical efficiencies. These effects lead to unreliable experimental results as well as loss of time and precious cell lines.

Unlike bacteria and fungi, *mycoplasma* contamination cannot be detected by visual inspection. They are small enough to pass through most sterilization filters; they are resistant to common antibiotics. Therefore, routine screening for *mycoplasma* contamination is essential.



Roche Applied Science offers *mycoplasma* detection kits to help detect contamination early.

Cross-Contamination

If different cell types are grown in the same laboratory, it is possible that cross-contamination can occur even when the strictest separation procedures are followed. It is well known that many cell lines are contaminated by HeLa cells. Cross-contamination by other cell lines cannot always be detected by microscopic examination. If a few cells of a faster growing cell type get into the culture, over a matter of months they will take over the culture. The change is gradual; you may not even notice it.



Establish identify tests criteria. For example, with human cell lines, STR (Short Tandem Repeats) profiling is a reliable method. Or for the easiest solution when in doubt, and if possible, replace the lines with a fresh, low-passage supply.

Vector DNA

General tips: Check the quality of your purified vector. Decide whether the sequences that support its normal function are suitable for your cellular system. To test the parameters of your system, always use a control vector that you know is functional.

Vector Integrity – The function of a vector depends on its structural integrity. Transfection efficiency can be influenced by the ratio of supercoiled form to relaxed form in the plasmid preparation, double-stranded breaks, nuclease degradation, and physical stresses arising from storage and handling.

Vector Preparation – Vectors are produced in bacterial systems and purified according to various protocols. Contaminants remaining in the vector preparation (e.g., CsCl, endotoxin) may influence transfection efficiency.

Vector Architecture (Promoter/Enhancer/ORI) –

Transfection systems are often optimized and compared using control vectors with strong viral regulatory elements (e.g., RSV, CMV, and SV40). However, the relative efficacy of viral promoter/enhancer systems can differ among cell lines by as much as two orders of magnitude. For example, in some cell lines, the SV40 system expresses the large T antigen (e.g., COS) highly efficiently due to autonomous plasmid amplification; in many other cell lines, the CMV promoter is more efficient.

In addition, expression rates of various CMV vectors can differ by more than an order of magnitude, in part due to the other regulatory elements in the vectors.

Transfection Protocol

General tips: Establish a suitable transfection protocol; begin with a standard protocol, then optimize it by varying the reagent / DNA ratio and amount of complex.

Preparation of the Transfection Complex – Variables such as transfection reagent / DNA ratio, ionic strength, buffer pH, and temperature affect the composition and function of the transfection complexes. Plasmid may be diluted in either sterile water or TE buffer. If you are using a commercial transfection reagent, carefully read the instructions that are supplied with the reagent. Trust the supplier; stick to their recommended protocols unless you have very good reasons for changing them. Prepare complex in medium that does not contain serum or other proteins; if the medium used for complex formation contains serum, it will inhibit transfection.

The optimal incubation time for complex formation can be quite critical and varies significantly for different transfection reagents.

Transfection Reagent / DNA Ratio (Charge Ratio) –

All transfection reagents are most efficient at a certain reagent / DNA ratio. Sometimes this ratio occurs within a quite narrow range; it must be optimized for each experimental system. The time it takes to find the optimal ratio is well invested. Not only is the ratio important, but the amount of complex added is often critical.


Diluent for forming complex – For most reagents it is critical to form the complex in plain basal medium or salt solution.




FuGENE® HD Transfection Reagent is an exception to this, since it allows complex to be formed in water, serum-free medium, or medium containing 10% serum.

If you are using water or medium containing 10% serum, make sure that it will work with your particular system.


Amount of Complex – There is an optimum amount of transfection reagent / DNA complex that should be applied to the cells. If you use too little, expression of the transfected DNA is weak; if you use too much, expression may decline due to cytotoxicity or other effects. The optimal amount usually must be determined experimentally.

 This can be clearly seen with FuGENE® HD Transfection Reagent.


 One exception occurs in transfections involving FuGENE® 6 Transfection Reagent, which gives good results over a wide dosage range. In many cases, this property of FuGENE® 6 Transfection Reagent makes dose optimization unnecessary.


The amount of complex required is related to the number of cells present. Fewer cells require less complex.

Application of the Transfection Complex – There are two alternative ways to apply the transfection complex to the cells: 1. add the concentrated complex dropwise to the medium, or 2. predilute the transfection complex with the medium, then perform a medium exchange. The first option is more convenient while the latter option provides more consistent application, which avoids localized toxic doses.

 For gentle reagents such as FuGENE® 6 Transfection Reagent and HD Transfection Reagents, you may place the complex in the container, then add freshly trypsinized cells.

Transfection Medium – The medium present during transfection could affect transfection efficiency either positively or negatively. This is true even for basic medium formulations, particularly when bovine serum is included. With a number of transfection reagents, efficiency is reduced in the presence of fetal bovine serum. Some companies provide optimized serum-free transfection media that may be used in combination with the transfection reagents. The transfection reagents provided by Roche Applied Science are effective in the presence or absence of serum.

 FuGENE® HD Transfection Reagent is unique in that it can transfect tumor cells maintained in 100% serum.

 Transfection efficiency may be reduced up to 25% if the cells are cultivated in the presence of antibiotics (e.g., penicillin, streptomycin, or fungizone). Thus, for transient transfections, we recommend using medium without antibiotics.

Timeline

General tips: Ensure a successful end result; set up a suitable timeline for your transfection experiment to ensure optimal expression of your protein of interest.

Start of Transfection – Twelve hours before transfection, split the cells into culture plates. At the start of transfection, the culture should be approximately 50-80% confluent, so they will be near confluency by the end of the experiment.

If serum is taken away during transfection, the cells may arrest for awhile.

The uptake of transfection complex by the cells is generally complete within 0.5-6 hours. After this time, there is no detectable increase in transfection efficiency.

Medium Exchange – Some transfection reagents require a medium change after the uptake period.

This step is essential if transfection must be performed in the absence of fetal bovine serum. With non-toxic transfection reagents, which work in the presence of serum (e.g., FuGENE® 6 Transfection Reagent or FuGENE® HD Transfection Reagent), this step can be eliminated if there is enough medium for the subsequent expression period.

If the complex is left on the cells until the time of assay, transfection efficiency increases for some cell lines, while in other cells there is no further increase after the first few hours.

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 during extended growth periods. This is especially important if the transfected cells are allowed to grow for 3-7 days, so they have time to reach maximum protein expression.

Time assay – Reporter gene expression is usually analyzed 24-48 hours after the start of transfection. Within this time span, the concentration of the reporter gene product generally increases; this increase depends on the strength of the promoter, cellular reactions involved in expressing the heterologous protein, health of the surviving cells, and nutritional factors.

If you are harvesting proteins for purification, it is more common to wait 3-7 days post transfection. At the time of harvest it is a good idea to include Complete Protease Inhibitor (e.g., Cat. Nos. 11697498001; 11836145001, available from Roche Applied Science) in the medium to prevent degradation.

Ordering Information

Product	Pack Size	Cat. No.
FuGENE® HD Transfection Reagent	0.4 ml (up to 120 transfections)	04709691001
	1.0 ml (up to 300 transfections)	04709705001
	5 x 1 ml (up to 1,500 transfections)	04709713001
FuGENE® 6 Transfection Reagent	1 ml (up to 300 transfections)	11814443001
	0.4 ml (up to 120 transfections)	11815091001
	5 x 1 ml (up to 1,500 transfections)	11988387001
	Custom-pack (150 ml minimum)	11988484001
X-tremeGENE Q2 Transfection Reagent	1.8 ml (450 transfections)	03036421001
	0.4 ml (100 transfections)	03045595001
X-tremeGENE siRNA Transfection Reagent	1 ml (400 transfections in a 24-well plate)	04476093001
	5 x 1 ml (2,000 transfections in a 24-well plate)	04476115001
DOTAP Liposomal Transfection Reagent	0.4 ml	11811177001
	2 ml (5 x 0.4 ml, 2 mg)	11202375001
DOSPER Liposomal Transfection Reagent	0.4 ml	11811169001
	2 ml (5 x 0.4 ml, 2 mg)	11781995001

NOTICE TO PURCHASER

Purchaser represents and warrants that it will use FuGENE® Transfection Reagent purely for research purposes. Transfected cells, materials produced, and any data derived from the use of FuGENE® Transfection Reagent, may be used only for the internal research of Purchaser whether Purchaser is a "for-profit" or a "not-for-profit" organization. Under no circumstances may FuGENE® Transfection Reagent be used by Purchaser or any third party for a commercial purpose unless Purchaser has negotiated a license for commercial use with Fugent, LLC (contact information: license@fugentllc.com). For purposes of the foregoing sentence, "commercial purpose" shall mean use of FuGENE® Transfection Reagent for profit or commercial gain. By using FuGENE® Transfection Reagent, Purchaser agrees to be bound by the above terms. If Purchaser wishes not to be bound by these terms, Purchaser agrees to return the FuGENE® Transfection Reagent to Roche Diagnostics for a full refund.

Trademarks

The ATCC trademark and trade name and any and all ATCC catalog numbers are trademarks of the American Type Culture Collection. FuGENE is a registered trademark of Fugent, L.L.C., USA. COMPLETE is a trademark of Roche. Other brands or product names are trademarks of their respective holders.

References

- [1] **Linda B. Jacobsen, Susan A. Calvin, Kim E. Colvin and MaryJo Wright.** FuGENE 6 Transfection Reagent: the gentle power. Special issue: Transfection of Mammalian Cells - Methods 33 (2004) 104-112; Edited by A. L. Peel;
- [2] **Bindu Raghavan, Guojuan Zhang, Mark Kotur, Jacquelyn Cheatham, and Joanne Trgovcich.** Superiority of FuGENE® HD Transfection Reagent in Minimizing Non-Specific Cellular Interferon Responses. Biochemica 3 (2006) 20-23.
- [3] **Vivien Nagy and Manfred Watzel.** FuGENE 6 Transfection Reagent: Minimizing Reagent-Dependent Side Effects as Analyzed by Gene-Expression Profiling and Cytotoxicity Assays. Biochemica 4 (2004) 9-11.
- [4] **Kouichi Hasegawa, Shin-ya Yasuda, and Hirofumi Suemori.** Superior Transfection of Human Embryonic Stem Cells with FuGENE® HD Transfection Reagent. Biochemica 4 (2006) 24-26.
- [5] **Susan Calvin, Jay Wang, Jeff Emch, Simone Pitz, and Linda Jacobsen.** FuGENE® HD Transfection Reagent: Choice of a Transfection Reagent with Minimal Off-Target Effect as Analyzed by Microarray Transcriptional Profiling. Biochemica 4 (2006) 27-30.

For a detailed Troubleshooting please see Application Note 2, Setting up transfection controls

For a complete overview of related products and manuals, please visit and bookmark our Special Interest Site www.roche-applied-science.com/transfection

ATCC®

To ensure the quality of cells to be transfected, Roche recommends using freshly-obtained, low-passaged cell lines from ATCC®. For more information, please visit and bookmark www.atcc.org



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