

January 2008

Attractene Transfection Reagent Handbook

For efficient DNA transfection of a broad range of cell lines, including sensitive cell types



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Kit Contents

Attractene Transfection Reagent	(0.5 ml)	(1 ml)	(4 x 1 ml)
Catalog no.	301004	301005	301007
Transfections per kit (in 24-well plates)	Up to 330	Up to 660	Up to 2640
Attractene Transfection Reagent	0.5 ml	1 ml	4 x 1 ml
Handbook	1	1	1

Shipping and Storage

Attractene Transfection Reagent is supplied as a ready-to-use solution and is shipped at ambient temperature without loss in stability. However, it should be stored at 2–8°C upon arrival. It is stable for 1 year at 2–8°C. Attractene Transfection Reagent does not need to be stored on ice during the transfection procedure.

Product Use Limitations

Attractene Transfection Reagent is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Product Warranty and Satisfaction Guarantee

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

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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 technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Safety Information

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

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of Attractene Transfection Reagent is tested against predetermined specifications to ensure consistent product quality.

Introduction

Attractene Transfection Reagent is the next generation in lipid technology, ideal for high-efficiency, low-cytotoxicity DNA transfection of all adherent cells, including sensitive cell types. It enables a broad range of applications, including transient and stable transfection, cotransfection, and shRNA-vector transfection.

Principle and procedure

Attractene Reagent is a nonliposomal lipid which enables highly efficient DNA transfection. Under a range of experimental conditions, including after storage of transfection complexes and dilution in cell-culture medium, Attractene Reagent consistently provides higher transfection efficiency than alternative reagents. This feature allows flexible handling and ease of use for many experimental setups such as using automated platforms and transfection at high- or low-throughput levels.

Using Attractene Reagent ensures exceptionally low cytotoxicity, which is critical for successful transfection experiments. Cytotoxic effects result in data that are difficult to interpret and unreliable. Stressed cells have altered gene expression patterns compared to unstressed cells. In addition, the presence of dead cells makes observation of changes in cell phenotype difficult. To ensure that results are due to the nucleic acid transfected and not the transfection process itself, cytotoxicity must be kept to a minimum.

Attractene Transfection Reagent can be used for a range of transfection applications, including transient or stable transfection. It enables efficient cotransfection of multiple DNA constructs. Attractene Reagent is a one-tube reagent, which reduces protocol steps and ensures ease of handling making it suitable for use with automated systems. Transfection of shRNA (short-hairpin RNA) vectors for gene silencing experiments can be achieved with high efficiency. Attractene Reagent also enables transfection in the presence of serum without lowering transfection efficiencies.

Cell-specific QIAGEN transfection protocols

The TransFect Protocol Database is an invaluable resource for transfection experiments. Simply visit www.qiagen.com/TransFect and then enter the cell type, nucleic acid, and plate format to receive a QIAGEN transfection protocol to print out or download in convenient PDF format. Use of the TransFect Protocol Database is free of charge and no registration is required.

Description of protocols

Two protocols are provided in this handbook: the Fast-Forward Protocol and the Traditional Protocol (Figure 1). In the Fast-Forward Protocol, plating and transfection of cells are performed on the same day. DNA is diluted in culture

medium without serum. Attractene Transfection Reagent is added to the diluted DNA to produce Attractene–DNA complexes. The cells are seeded and then complexes are added directly to the freshly seeded cells.

In the Traditional Protocol, cells are plated 24 hours prior to transfection. Cells are seeded in culture medium containing serum and antibiotics the day before transfection and incubated under normal growth conditions. The next day, DNA is diluted in culture medium without serum. Attractene Transfection Reagent is added directly to the diluted DNA to produce Attractene–DNA complexes. During complex formation, the medium on the cells is changed. Next, the complexes are added to the cells.

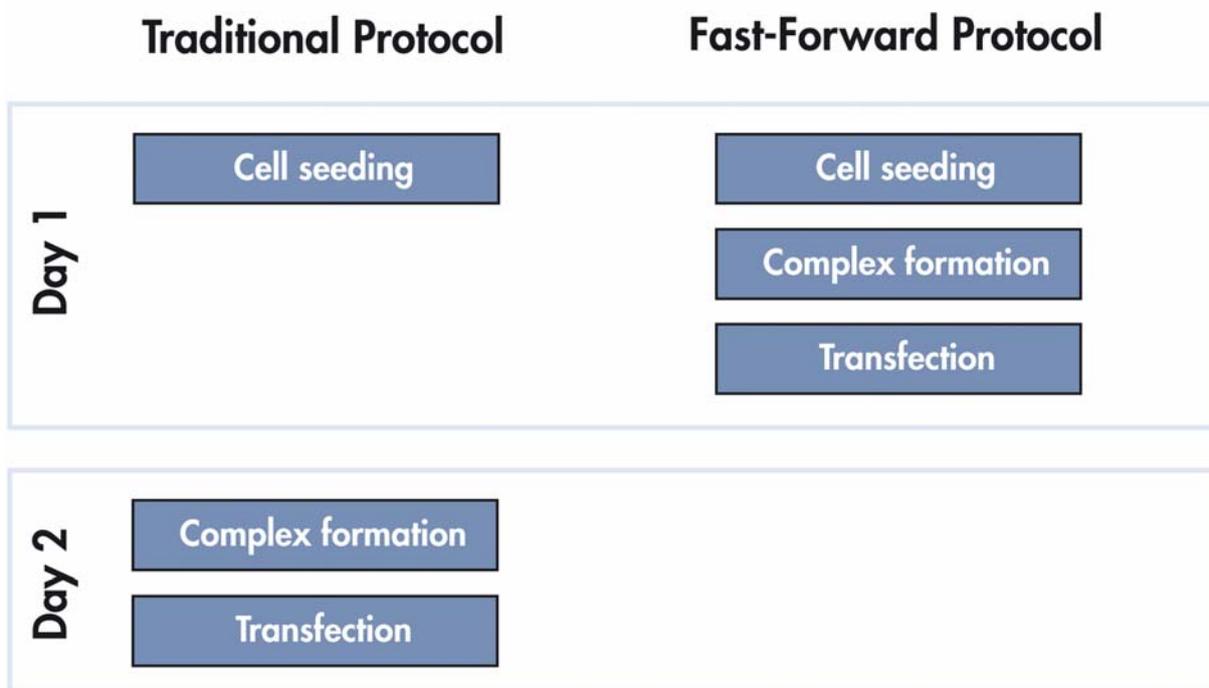


Figure 1. The Fast-Forward Protocol saves time and labor. In the Fast-Forward Protocol, cell seeding, complex formation, and transfection are all performed on the same day. In the Traditional Protocol, cell seeding is performed the day before transfection.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- Culture medium
- DNA of interest
- Cells of interest

Important Notes

Optimizing DNA and Attractene Transfection Reagent amounts

To achieve optimal transfection results for a given cell line/DNA combination, the amount of DNA and Attractene Reagent should be optimized. Table 1 shows a pipetting scheme for optimizing transfection of cells in 24-well plates. This scheme shows 9 different conditions which we recommend to test when optimizing transfection (i.e., when determining the conditions that provide highest transfection efficiency and/or lowest cytotoxicity). As a starting point, we recommend using 0.4 μg DNA and 1.5 μl Reagent (in bold in Table 1). For transfection using other culture formats, refer to Tables 3 and 4, pages 12 and 14 respectively.

Table 1. Pipetting scheme for optimizing transfection in a 24-well plate*

Amount of DNA	0.2 μg	0.2 μg	0.2 μg
Volume of Attractene Reagent	0.5 μl	0.75 μl	1.5 μl
Amount of DNA	0.4 μg	0.4 μg	0.4 μg
Volume of Attractene Reagent	1 μl	1.5 μl	3 μl
Amount of DNA	0.6 μg	0.6 μg	0.6 μg
Volume of Attractene Reagent	1.5 μl	2.25 μl	4.5 μl

The recommended starting point for transfection is shown in bold.

* Amounts given are per well of a 24-well plate.

Optimizing transfection in different formats

Tables 3 (page 12) and 4 (page 14) give starting points for optimization of transfection in different formats for the Fast-Forward Protocol and the Traditional Protocol, respectively.

Preparing a master mix for multiple transfections

If performing multiple transfections in parallel, a transfection complex master mix should be prepared for distribution into the plate wells. Prepare 10% more master mix than is required, or at least enough for 1 extra sample, to allow for pipetting errors. Keep the overall amounts and volumes, as given in Tables 3 and 4, constant. Prepare a dilution of the DNA in medium (without serum and antibiotics), add Attractene Reagent, and continue as indicated in the protocols.

For example, calculate the components of the transfection complex master mix for transfection in 24-well plates as follows:

DNA: (number of replicates+1) x 0.4 μ g

Medium: (number of replicates+1) x 60 μ l

Attractene Reagent: (number of replicates+1) x 1.5 μ l

For the Traditional Protocol:

Medium added to the complexes: (number of replicates + 1) x 500 μ l

Cell density at transfection

The optimal cell confluency for transfection should be determined for every new cell type to be transfected and kept constant in future experiments. This is achieved by counting the cells before seeding and, in the case of the Traditional Protocol, by keeping the interval between seeding and transfection constant. This ensures that cell density is not too high and the cells are in the optimal physiological condition at transfection. For adherent cells, the optimal confluency at the time of complex addition is normally 40–80%.

The recommended number of cells to seed for different formats for the Fast-Forward Protocol and the Traditional Protocol is shown in Table 2. These cell numbers can be used as starting points for transfection optimization. Cell densities recommended for the Fast-Forward Protocol are usually 2–3 times higher than those recommended for the Traditional Protocol. However, the optimal cell number will be different for different cell types; for example small-sized cells, such as HEK 293, should be transfected at higher cell numbers than larger-sized cells.

Table 2. Recommended number of cells per culture vessel

Culture format	Fast-Forward Protocol	Traditional Protocol
96-well plate	1–8 x 10 ⁴	0.5–4 x 10 ⁴
24-well plate	0.4–1.6 x 10 ⁵	2–8 x 10 ⁴
6-well plate	1.6–8 x 10 ⁵	0.8–4 x 10 ⁵
100 mm dish	1–5 x 10 ⁶	0.5–2.5 x 10 ⁶

Protocol: Fast-Forward Transfection of Cells with DNA in 24-Well Plates

The following protocol is provided as a starting point for optimization of transfection of cells in 24-well plates without preplating of cells 24 h prior to transfection. The amounts given are for one well of a 24-well plate. For transfection using other culture formats, refer to Table 3, page 12.

Procedure

- 1. Dilute 0.4 μg DNA dissolved in TE buffer, pH 7–8 (minimum DNA concentration: 0.1 $\mu\text{g}/\mu\text{l}$) with medium without serum, proteins, or antibiotics, to a total volume of 60 μl .**

For example, if the DNA concentration is 1 $\mu\text{g}/\mu\text{l}$, dilute 0.4 μl DNA in 59.6 μl medium.

- 2. Add 1.5 μl Attractene Transfection Reagent. Mix by pipetting up and down or vortexing. Centrifuge for a few seconds to remove any liquid from the top of the tube if necessary.**
- 3. Incubate the samples for 10–15 min at room temperature (15–25°C) to allow transfection complex formation. Continue with steps 4 and 5 during the incubation time.**

Note: Transfection complex formation takes a minimum of 10–15 min. The transfection complexes will remain stable during the time it takes to prepare the cells for transfection. However, the incubation time should not be extended for longer than is necessary for cell preparation.

- 4. Harvest the cells by trypsinization and suspend in culture medium containing serum and antibiotics.**

Note: The cells should be healthy and in logarithmic growth phase.

It is important that serum and antibiotics are present in the culture medium at this point because transfections are performed without changing the medium. The cultivation of cells over this time without serum would deprive the cells of essential growth factors. This does not apply to cells that are routinely cultivated without serum.

- 5. Count the cells in the harvested cell suspension and adjust the cell density to 0.4–1.6 $\times 10^5$ cells in 500 μl (depending on the cell line). The optimal cell density should be determined for each cell line.**

Note: For example, cell density should be adjusted to 0.8–3.2 $\times 10^5$ cells per ml for a final cell density at transfection of 0.4–1.6 $\times 10^5$ cells in 500 μl .

As plating and transfection of cells are carried out on the same day, higher cell densities are required than would be necessary if the cells had a longer incubation time prior to transfection. As a guideline, the required cell

number is usually 2–3 fold higher than that used for the Traditional Protocol (where cells are plated on the day before transfection).

- 6. Add 500 μ l of the cell suspension to a well of a 24-well plate. Next, add the transfection complexes to the well. Mix by pipetting up and down twice.**
- 7. Incubate the cells with the transfection complexes under their normal growth conditions (typically 37°C and 5% CO₂).**

In most cases, removal of transfection complexes is not necessary. However, if cytotoxicity is observed, remove the complexes after 6–18 hours and add fresh culture medium.

- 8. Assay the cells for expression of the transfected gene after an appropriate incubation time. The length of the incubation time depends on the assay and the transfected gene.**
- 9. For stable transfections, passage the cells into the appropriate selection medium 24–48 h after transfection. Maintain the cells in selective culture medium until colonies appear.**

Table 3. Starting points for transfection in different formats using the Fast-Forward Protocol

Culture format	DNA amount (μg)	Final volume of diluted DNA (μl)	Attractene Reagent (μl)	Volume of cell suspension (μl)
Protocol step	1	1	2	6
96-well plate	0.2	50	0.75	100
24-well plate	0.4	60	1.5	500
6-well plate	1.2	100	4.5	2000
100 mm dish	4	300	15	10,000

Protocol: Transfection of Cells with DNA in 24-Well Plates (Traditional Protocol)

The following protocol is provided as a starting point for optimization of transfection of cells in 24-well plates. In this protocol, cells are plated 24 h prior to transfection. The amounts given are for one well of a 24-well plate. For transfection using other culture formats, refer to Table 4, page 14. This protocol can be used for sensitive cell lines.

Procedure

- 1. The day before transfection, seed $2\text{--}8 \times 10^4$ cells (depending on the cell type) in $500 \mu\text{l}$ of an appropriate culture medium containing serum and antibiotics.**
- 2. Incubate the cells under normal growth conditions (typically 37°C and $5\% \text{CO}_2$).**

Cells should be 40–80% confluent on the day of transfection.
- 3. On the day of transfection, dilute $0.4 \mu\text{g}$ DNA dissolved in TE buffer, pH 7–8 (minimum DNA concentration: $0.1 \mu\text{g}/\mu\text{l}$) with medium without serum, proteins, or antibiotics to a total volume of $60 \mu\text{l}$.**
- 4. Add $1.5 \mu\text{l}$ Attractene Transfection Reagent to the DNA solution. Mix by pipetting up and down or vortexing. Centrifuge for a few seconds to remove any liquid from the top of the tube if necessary.**
- 5. Incubate the samples for 10–15 min at room temperature ($15\text{--}25^\circ\text{C}$) to allow complex formation.**
- 6. While complex formation takes place, gently aspirate the medium from the cells and add $500 \mu\text{l}$ fresh medium (containing serum and antibiotics) to the cells.**
- 7. Add the transfection complexes drop-wise onto the cells. Gently swirl the plate to ensure uniform distribution of the transfection complexes.**
- 8. Incubate the cells under their normal growth conditions and analyze the cells after an appropriate time.**

In most cases, removal of transfection complexes is not necessary. However, if cytotoxicity is observed, remove the complexes after 6–18 hours and add fresh culture medium.
- 9. For stable transfections, passage the cells into the appropriate selection medium 24–48 h after transfection. Maintain the cells in selective culture medium until colonies appear.**

Table 4. Starting points for transfection in different formats using the Traditional Protocol

Culture format	DNA amount (μg)	Final volume of diluted DNA (μl)	Attractene Reagent (μl)	Volume of medium* added to cells (μl)
Protocol step	3	3	4	6
96-well plate	0.2	50	0.75	100
24-well plate	0.4	60	1.5	500
6-well plate	1.2	100	4.5	2000
100 mm dish	4	300	15	10,000

* Culture medium containing serum and antibiotics.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

Comments and suggestions

Low transfection efficiency

- | | |
|--|---|
| a) Suboptimal reagent to DNA ratio | If the ratio of transfection reagent to plasmid DNA is suboptimal, the overall charge of the complexes may be negative, neutral, or strongly positive, which can lead to inefficient adsorption to the cell surface. Optimize the Attractene Transfection Reagent to DNA ratio using Table 1, page 9. |
| b) Insufficient Attractene Reagent–DNA complex | If the transfection efficiency is lower than expected and cytotoxicity is acceptably low, increase the overall amount of Attractene Reagent–DNA complex added to the cells. See the pipetting scheme in Table 1, page 9. |
| c) Suboptimal incubation time | Different cell types achieve maximal expression levels at different times after 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. |
| d) Suboptimal cell density | If cell density at the time of adding Attractene Reagent–DNA complexes is not at an optimal level, this can lead to insufficient uptake of complexes into the cells. For adherent cells, the optimal confluency for DNA transfection is normally 40–80%. Be sure to seed cells a minimum of 24 h before transfection if using the Traditional Protocol. Usually higher cell numbers are required for the Fast-Forward Protocol in order to compensate for the reduced doubling time between plating and transfection. |

Comments and suggestions

- e) 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.
- f) Poor DNA quality DNA should be of high quality, as impurities can lower transfection efficiency. Plasmid DNA should be purified using HiSpeed[®], QIAfilter, or QIAGEN Plasmid Kits. For endotoxin-sensitive cells, we recommend using EndoFree[®] Plasmid Kits (see ordering information, page 20).

Excessive cell death

- a) Excessive exposure of cells to Attractene Reagent–DNA complexes If sensitive cells demonstrate extensive cell death after treatment with Attractene Reagent–DNA complexes, remove the complexes after 6 h and add fresh medium.
- b) Concentration of Attractene Reagent–DNA complexes too high Decrease the amount of Attractene Reagent–DNA complexes added to cells, but keep the ratio of Attractene Reagent to DNA constant.
- c) Cells are stressed Avoid stressing cells with temperature shifts and long periods without medium during washing steps. Ensure that cell density is not too low at transfection. For adherent cells, the optimal confluency for transfection is 40–80%. Be sure to seed cells a minimum of 24 h before transfection if using the Traditional Protocol. Usually higher cell numbers are required for the Fast-Forward Protocol in order to compensate for the reduced doubling time between plating and transfection. We recommend performing the transfection in the presence of serum so that the cells are not deprived of necessary growth factors and nutrients during this long incubation.

Comments and suggestions

- d) Poor DNA quality DNA should be of high quality, as impurities can lower transfection efficiency. Plasmid DNA should be purified using HiSpeed, QIAfilter, or QIAGEN Plasmid Kits. For endotoxin-sensitive cells, we recommend using EndoFree Plasmid Kits (see ordering information, page 20).

Variable transfection efficiencies in replicate experiments

- a) Inconsistent cell confluencies in replicate experiments Count cells before seeding to ensure that the same number of cells is seeded for each experiment. Keep incubation times between replicate seeding and complex addition consistent between experiments. Cells should be seeded at least 24 h before transfection if using the Traditional Protocol.
- b) 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.
- c) Cells have been passaged too many times Cells that have been passaged a large number of times tend to change their growth behavior, morphology, and potential for transfection. 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 a low passage number (<50 splitting cycles).
- d) Serum variability Variations in serum quality can lead to differences in transfection efficiency. It is advisable to test a small lot of serum from a reputable supplier in a control experiment. Once a given lot has yielded satisfactory and reproducible results, sera from this lot should be used for further experiments.

Difficulty isolating cell clones stably expressing DNA

- a) Suboptimal concentration of the selection reagent The optimal concentration of a given selection reagent is dependent on the cell density and the cell type. This should be evaluated for each cell type.

Comments and suggestions

- | | |
|--|--|
| b) Suboptimal incubation time between transfection and selection | Ensure that the incubation time between transfection and the start of selection is long enough to allow optimal expression of the resistance gene used for selection. |
| c) Culture consists of a cell mixture | Ensure that the clones consist of single cells stably expressing DNA and not a mixture of stably expressing clones and resistant clones which do not express the DNA. The latter case may lead to loss of stably expressing cells over time. |

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

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

Ordering Information

Product	Contents	Cat. no.
Attractene Transfection Reagent (0.5 ml)	Attractene Transfection Reagent for up to 330 transfections in 24-well plates	301004
Attractene Transfection Reagent (1 ml)	Attractene Transfection Reagent for up to 660 transfections in 24-well plates	301005
Attractene Transfection Reagent (4 x 1 ml)	Attractene Transfection Reagent for up to 2640 transfections in 24-well plates	301007
Accessories		
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QIAfilter Plasmid Midi Kit (25)*	25 QIAGEN-tip 100, Reagents, Buffers, 25 QIAfilter Midi Cartridges	12243
QIAGEN Plasmid Mini Kit (25)*	25 QIAGEN-tip 20, Reagents, Buffers	12123
EndoFree Plasmid Maxi Kit (10)	10 QIAGEN-tip 500, Reagents, 10 QIAfilter Maxi Cartridges, Endotoxin-free Buffers	12362

* Larger prep and kit sizes available. Find out more at www.qiagen.com.

Notes

Notes

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