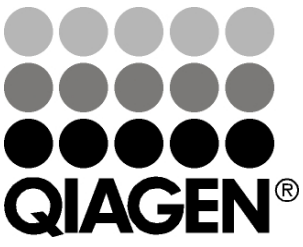
September 2012

TransMessenger® Transfection Reagent Handbook

For transfection of eukaryotic cells with RNA and siRNA



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Contents

Kit Contents

TransMessenger Transfection Reagent	**(0.5 ml)**
Catalog no.	**301525**
Number of transfections	**60–80***
TransMessenger Transfection Reagent (1 mg/ml)	0.5 ml
Enhancer R (1 mg/ml)	0.5 ml
Buffer EC	15 ml
Quick-Start Protocol	1

* Number of transfections depends on plate size: 60 transfections in 6-well plates or 80 transfections in 12-well plates.

Storage

TransMessenger Transfection Reagent, Enhancer R, 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. The solutions are stable for 1 year at 2–8°C. In contrast to many liposome-based reagents, TransMessenger Transfection Reagent is not sensitive to oxygen, and so does not require storage under an inert gas. Additionally, TransMessenger Transfection Reagent does not need to be stored on ice during the transfection procedure.

Intended Use

The TransMessenger Transfection Reagent is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS 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 the TransMessenger Transfection Reagent is tested against predetermined specifications to ensure consistent product quality.

Introduction

TransMessenger Transfection Reagent has been designed to efficiently transfect eukaryotic cells with RNA, including siRNA (see page 12). This handbook contains guidelines and protocols for the transfection of single-stranded RNA (see "Optimizing RNA transfection", page 10 and protocol on page 18) and double-stranded siRNA (pages 12–17).

The TransMessenger principle

TransMessenger Transfection Reagent is based on a lipid formulation and is used in conjunction with a specific RNA-condensing reagent (Enhancer R) and an RNA-condensing buffer (Buffer EC). In the first step of TransMessenger–RNA complex formation, the RNA is condensed by interaction with Enhancer R in a defined buffer system. In the second step, TransMessenger Transfection Reagent is added to the condensed RNA to produce TransMessenger–RNA complexes. The TransMessenger–RNA complexes are then mixed with serum-free medium and added directly to cells.

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 safety data sheets (SDSs), available from the product supplier.

- Culture plates
- Culture medium, serum, and antibiotics
- Incubator for culture growth
- Tubes
- Sterile phosphate-buffered saline solution (PBS) (1x PBS: 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). Store at room temperature (15–25°C).

Important Notes

General considerations for RNA transfection

Transfection efficiencies are affected by a variety of parameters. The following factors should be considered carefully.

Cell culture

A healthy cell culture lays the foundation for successful transfection, particularly when transfecting cells with RNA. We strongly recommend subculturing cells for a minimum of 24 hours before transfection. This ensures normal cell metabolism and increases the likelihood of RNA uptake. Best results are obtained using the confluence levels indicated in the appropriate protocol sections. Contamination with bacteria (e.g., mycoplasma) and fungi should be avoided, since this can drastically alter transfection results. Different cells or cell types have very specific medium, serum, and supplement requirements. We recommend using cells with a low passage number (<50 splitting cycles) to ensure that the cell genotype does not become altered.

Serum

In general, the use of serum enhances transfection. However, to avoid contamination with ribonucleases (RNases), we do not recommend using serum during transfection of RNA.

RNA structure

The structure of the transfected RNA molecule can influence the efficiency of transfection by influencing the formation of the TransMessenger Reagent–RNA complex. In addition, the presence of specific RNA characteristics, such as a cap, poly-A tail, or internal ribosomal entry site (IRES), may increase or decrease the efficiency of translation in a given cell type.

Messenger RNA (mRNA) species in eukaryotic cells usually terminate at the 5'-end with a cap and at the 3'-end with a poly-A tail. A cap is a modified nucleotide incorporated at the 5'-end of RNA transcripts that enhances mRNA stability and is necessary in some cell types to ensure optimal translation. A cap can be incorporated into an in vitro-transcribed RNA by adding an RNA cap-structure analog to the transcription reaction. A poly-A tail, a stretch of adenylate residues added 3' to the mRNA coding sequence, also stabilizes mRNA. A poly-A tail can be incorporated into an in vitro-transcribed RNA by using a relevant DNA template or added post-transcriptionally using a poly-A polymerase. An IRES is a specific sequence which helps the ribosome to access the mRNA, thereby enhancing

translation. However in some cell types, RNA species containing an IRES will not be efficiently translated due to a lack of factors necessary for translation initiation at an IRES in these cells.

RNA quality

Optimal transfection results are achieved when RNA of the highest purity is used for transfection. Therefore, only RNA of the highest quality, which is free of contaminating DNA and proteins, should be used. RNA purified with RNeasy® Kits and Oligotex® mRNA Kits is highly recommended for transfection.

We strongly recommend checking RNA quality before starting the transfection. RNA concentration and purity can be determined by measuring the absorbance at 260 nm (A_{260}) and 280 nm (A_{280}) using a spectrophotometer. An absorbance of 1 unit at 260 nm corresponds to ~40 µg RNA per milliliter. RNA integrity and size can be checked by denaturing agarose-gel electrophoresis and ethidium bromide staining. The RNA should appear as a sharp band on the stained gel. If the band is not sharp but appears as a smear containing smaller sized RNAs, it is likely that the RNA has suffered major degradation during its preparation. The functionality of the transcript can be determined by in vitro translation.

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that do not generally require cofactors to function. Since RNases are difficult to inactivate and minute amounts are sufficient to destroy RNA, do not use any plasticware, glassware, or solutions without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases during the transfection procedure. To create and maintain an RNase-free environment when working with RNA, the precautions listed below must be followed throughout the procedure.

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds, and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin. Change gloves frequently and keep tubes closed. Ensure that all glassware, plasticware, and solutions (including water and culture medium) are RNase-free.

Disposable plasticware

The use of sterile, disposable, plastic tubes throughout the procedure is recommended. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.

DNA contamination

No currently available purification method can guarantee that RNA is completely free of DNA, even when no DNA is visible on an agarose gel. For RNA transfection, digestion of the purified RNA with RNase-free DNase is recommended. The RNA can then be repurified using the RNeasy Kit.

Optimizing RNA transfection

To achieve optimal transfection efficiency for every cell type–RNA combination, we recommend optimizing a number of parameters:

- The amounts of TransMessenger Transfection Reagent, RNA, and TransMessenger–RNA complexes
- The cell number/confluency
- The length of exposure of cells to TransMessenger–RNA complexes

Once the parameters yielding maximum transfection efficiency have been determined, they should be kept constant in every experiment using a particular cell type/RNA combination.

Cell density at transfection

Table 1 lists the recommended number of adherent cells to seed per culture well the day prior to transfection of single-stranded RNA. The optimal confluency for transfection of adherent cells with single-stranded RNA is 80–90%, while the optimal confluency for transfection of adherent cells with siRNA is 50–80%. The optimal confluency should be determined for every new cell type 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 (minimum 24 hours) constant. This will ensure that the cell density is not too high and that the cells are in optimal physiological condition on the day of transfection.

Table 1. Recommended number of cells to seed the day before transfection of single-stranded RNA

Culture format	Recommended no. of adherent cells to seed*
96-well plate	2–3 x 10^4
48-well plate	4–8 x 10^4
24-well plate	8–10 x 10^4
12-well plate	2–3 x 10^5
6-well plate	4–6 x 10^5

* Be sure that cells are seeded a minimum of 24 hours before transfection of RNA. Actual cell number used depends on cell type and size. Sufficient cells should be seeded such that the culture is 80–90% confluent on the day of transfection. The volume of medium used is not critical. Use a volume suitable for the cell culture format.

Amount of RNA

The amount of RNA used is a critical factor for efficient transfection. Toxic effects may arise if too much RNA is used, while conversely, expression levels may be too low if insufficient RNA is used. Therefore, the amount of RNA transfected should be optimized for every new RNA and/or new cell type used. The recommended amount of RNA for transfection using 6-well plates is 2 µg. A pipetting scheme for optimizing the transfection of adherent cells in 6-well plates is provided in Table 2, page 12. For transfection using other culture formats, refer to Table 3, page 15.

Amount of Enhancer R

The RNA (µg) to Enhancer R (µl) ratio of 1:2 given in the pipetting scheme in Table 2, page 12, should not be changed. Efficient condensation of RNA with Enhancer R is determined by the mass quantity of RNA.

Ratio of TransMessenger Transfection Reagent to RNA–Enhancer R mixture

The overall charge of the TransMessenger–RNA complex is determined by the ratio of TransMessenger Transfection Reagent to RNA–Enhancer R mixture. Optimal binding of TransMessenger–RNA complexes to negatively charged groups (e.g., sialylated glycoproteins) on the cell surface requires a slightly positive net charge. The ratio of TransMessenger Transfection Reagent (µl) to RNA (µg) is an important factor to optimize for every new cell type and RNA used. As a starting point for optimization we recommend using an RNA:TransMessenger Transfection Reagent ratio of 2 µg RNA to 8 µl TransMessenger Reagent when using 6-well plates.

A pipetting scheme for optimizing the transfection of adherent cells in 6-well plates is provided in Table 2. To optimize transfection in other culture formats, prepare separate transfection mixtures using:

- The starting point RNA and TransMessenger Transfection Reagent quantities listed in Table 3, page 15.
- Roughly half the listed ratios and quantities.
- Double the listed ratios and quantities.

Incubation period with TransMessenger–RNA complexes

Remove TransMessenger–RNA complexes 3 hours after their addition to the cells, wash the cells with PBS, and add fresh medium. If no cytotoxic effects are observed, the incubation time can be increased up to 4 hours.

Table 2. Pipetting scheme for optimizing the transfection of adherent cells in 6-well plates

Amount of RNA	Ratio of RNA to TransMessenger Transfection Reagent		
	1:2	**1:4**	**1:8**
1 µg	1 µg RNA 2 µl Enhancer R 2 µl TransMessenger Reagent	1 µg RNA 2 µl Enhancer R 4 µl TransMessenger Reagent	1 µg RNA 2 µl Enhancer R 8 µl TransMessenger Reagent
2 µg	2 µg RNA 4 µl Enhancer R 4 µl TransMessenger Reagent	2 µg RNA 4 µl Enhancer R 8 µl TransMessenger Reagent	2 µg RNA 4 µl Enhancer R 6 µl TransMessenger Reagent
4 µg	4 µg RNA 8 µl Enhancer R 8 µl TransMessenger Reagent	4 µg RNA 8 µl Enhancer R 16 µl TransMessenger Reagent	4 µg RNA 8 µl Enhancer R 32 µl TransMessenger Reagent

RNA interference (RNAi), siRNA, and TransMessenger Transfection Reagent

Scientists at QIAGEN have successfully performed RNAi experiments using TransMessenger Transfection Reagent and a gene-specific siRNA (1). Below are some guidelines based on information from current literature (2–5), and our own experiences.

Controls for RNAi experiments

Controls should always be included in RNAi experiments. An siRNA with a scrambled oligonucleotide sequence can be used as a negative control. Library siRNAs that are directed against human gene products and have been shown to deliver gene-specific silencing in RNAi experiments are available from the QIAGEN siRNA Oligonucleotide Synthesis Service (see page Error! Bookmark not defined. for ordering information). Fluorescently labeled siRNA allows transfection efficiency to be easily followed.

Designing an siRNA

The design of an siRNA is a critical factor in its ability to mediate gene-specific silencing. QIAGEN provides an siRNA oligonucleotide synthesis service that offers expert advice on the design of an siRNA to achieve optimal gene-silencing effects. For more information visit www.qiagen.com/sirna.

Measuring the gene-silencing effect

The gene-silencing effect can be monitored at the protein level by western blotting, immunofluorescence, gene functional analysis, or BD FACS®. Silencing can also be monitored at the mRNA level by real-time RT-PCR. QIAGEN offers the QuantiFast® SYBR® Green RT-PCR Kit and the QuantiFast Probe RT-PCR Kit for highly sensitive, specific and fast one-step real-time RT-PCR analysis. All expression data should be compared to levels of a reference gene to exclude the possibility of non-specific effects.

General recommendations

The guidelines and recommendations below are based on RNAi experiments performed in 24-well plates. Please keep in mind that these are only guidelines, and the efficiency of transfection can be dependent on many different parameters, such as siRNA quality, cell type, passage number, and confluency of the cells at the time of transfection. Variations of these parameters should be considered if varying the amounts and ratios of siRNA and TransMessenger Reagent used does not lead to the desired results. If you would like to discuss optimization of your conditions, please contact QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

RNAi information online

The QIAGEN Transfection Tools web site contains siRNA transfection protocols and the most up-to-date information and literature on QIAGEN reagents for RNAi. Visit the Transfection Tools site for answers to your RNAi questions — www.qiagen.com/transfectiontools/.

Optimizing siRNA transfection

To achieve the best results in siRNA transfection we recommend optimizing the following parameters.

Amount of siRNA and Enhancer R

The amount of siRNA used is a critical factor for efficient transfection and gene silencing. The recommended starting amount for transfection of siRNA in 24-well plates is 0.8 µg. A pipetting scheme for optimizing the transfection of siRNA in adherent cells in a 24-well format is provided in Table 4 on page 20. Always use a 1:2 ratio of nucleic acid (µg) to Enhancer R (µl).

Ratio of TransMessenger Transfection Reagent to siRNA

The ratio of TransMessenger Transfection Reagent to siRNA should be optimized for every new cell type and siRNA combination used. As a starting point for optimization we recommend using an siRNA to TransMessenger Transfection Reagent ratio (µg: µl) of 1:5 when using 24-well plates.

To optimize siRNA transfection in 24-well format, prepare separate transfection mixtures using:

- The starting point siRNA and TransMessenger Reagent quantities that are given in the protocol on page 15.
- Half the listed quantities and ratios.
- Double the listed ratios and quantities.

Cell density at transfection

The optimal confluency for transfection of adherent cells with siRNA is 50–80%. The optimal confluency should be determined for every new cell type 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 (minimum 24 hours) constant. This will ensure that the cell density is not too high and that the cells are in optimal physiological condition on the day of transfection.

Table 3. Pipetting scheme for optimization of siRNA transfection in 24-well plates

Amount of RNA	Ratio of RNA to TransMessenger Transfection Reagent		
	1:2.5	**1:5**	**1:10**
0.4 µg	0.4 µg RNA 0.8 µl Enhancer R 1 µl Trans-Messenger Reagent	0.4 µg RNA 0.8 µl Enhancer R 2 µl Trans-Messenger Reagent	0.4 µg RNA 0.8 µl Enhancer R 4 µl Trans-Messenger Reagent
0.8 µg	0.8 µg RNA 1.6 µl Enhancer R 2 µl Trans-Messenger Reagent	0.8 µg RNA 1.6 µl Enhancer R 4 µl Trans-Messenger Reagent	0.8 µg RNA 1.6 µl Enhancer R 8 µl Trans-Messenger Reagent
1.6 µg	1.6 µg RNA 3.2 µl Enhancer R 4 µl Trans-Messenger Reagent	1.6 µg RNA 3.2 µl Enhancer R 8 µl Trans-Messenger Reagent	1.6 µg RNA 3.2 µl Enhancer R 16 µl Trans-Messenger Reagent

Guidelines for transfection of siRNA duplexes using TransMessenger Transfection Reagent

The procedure below is based on the TransMessenger Reagent standard procedure and RNAi studies using targets described by Elbashir et al (2). This procedure is provided as a starting point for optimization of siRNA transfection in mammalian cells using TransMessenger Transfection Reagent. For specific cell type and targets, optimal conditions could be different from those described here. Be sure to read the background information and the protocol notes in “Optimizing siRNA transfection“, page 14, before starting.

1. **The day before transfection, seed 50,000 to 100,000 cells (depending on cell type and the time point of analysis) per well of a 24-well plate in 0.5 ml appropriate growth medium containing serum and antibiotics.**

   Make sure that cells are in good condition and are seeded 24 h before transfection. Cells should be 50–80% confluent on the day of transfection.

2. **Incubate cells under their normal growth conditions (typically 37°C and 5% CO_2).**
3. **On the day of transfection, dilute 1.6 µl Enhancer R in Buffer EC. Add 0.8 µg siRNA (minimum siRNA concentration, 0.1 µg/µl) and mix by vortexing. The final volume should be 100 µl.**

   IMPORTANT: Always mix Enhancer R with Buffer EC before adding RNA. Always keep the ratio (µg: µl) of siRNA to Enhancer R constant (1:2).
4. **Incubate at room temperature (15–25°C) for 5 min, and then centrifuge the mixture for a few seconds to collect drops from the top of the tube.**
5. **Add 4 µl TransMessenger Transfection Reagent to the siRNA–Enhancer R mixture. Mix by pipetting up and down 5 times, or vortexing for 10 s.**
6. **Incubate the samples for 10 min at room temperature to allow transfection–complex formation.**
7. **While complex formation is taking place, gently aspirate the growth medium from the plate, and carefully wash cells once with sterile PBS using 1.5–2 times the volume of media used for cell seeding.**

   IMPORTANT: Do not allow the cells to become dry. Minimize the time they are without medium.
8. **Add 300 µl cell growth medium (without serum or antibiotics) to the tube containing the siRNA–TransMessenger Reagent complexes. Mix by pipetting up and down twice, and then immediately add the transfection complexes drop-wise onto the cells. Gently swirl the plate to ensure uniform distribution of the transfection complexes.**

   Note: Use of media without serum or antibiotics avoids the potential introduction of RNases. It is possible that transfection in the presence of serum may give improved results. Double-stranded siRNA is more resistant to degradation by RNAses than single-stranded RNA and therefore, if RNase contamination is not a concern, or if your serum is validated to have no detectable RNase activity, medium containing serum can be used.
9. **Incubate cells with the transfection complexes for 3 h under their normal growth conditions.**

   Note: If no cytotoxic effects are observed, the incubation time can be increased up to 4 h.

10. **Remove complexes from the cells, wash cells once with PBS, and then add 500 µl fresh medium containing serum and antibiotics to the cells.**
11. **Incubate cells under their normal growth conditions and monitor gene silencing after an appropriate incubation time. Change medium as required.**

    Note: The optimal time point for gene silencing analysis is dependent on cell type, the gene targeted, and method of analysis. A time course experiment should be performed to determine the appropriate incubation time.

Protocol: Transfection of Adherent Cells with RNA

The following protocol is for transfection of adherent cells using single-stranded RNA in one well of a 6-well plate. For transfection of siRNA, see page 14. As a starting point, use 2 µg RNA for transfection in 6-well plates. Starting points for optimizing transfection in other formats are listed in Table 3 on page 15. See Table 1 on page 11 for the recommended number of cells to seed. Optimal transfection conditions should be determined for every cell type–RNA combination to obtain the highest transfection efficiency with TransMessenger Transfection Reagent. Refer to the optimization guidelines starting on page 15.

Important notes before starting

- We strongly recommend reading this handbook carefully before starting.
- Check the integrity and functionality of the RNA (see "RNA quality", page 9).
- The RNA should be capable of being efficiently translated in the cell type used for transfection (e.g., IRES compatibility; see "RNA structure", page 8).
- The cells should be in optimal physiological condition on the day of transfection. Subculture the cells a minimum of 24 hours before transfection. The optimal confluency for transfection is 80–90%.

Procedure

1. **The day before transfection, seed 4–6 x 10^5 cells (depending on the cell type) per well of a 6-well plate in 2 ml appropriate growth medium containing serum and antibiotics.**

   Note: Make sure that cells are in good condition and are seeded at least 24 h before transfection. Cells should be 80–90% confluent on the day of transfection.

2. **Incubate cells under their normal growth conditions (generally 37°C and 5% CO_2).**

3. **On the day of transfection, dilute 4 µl Enhancer R in Buffer EC. Add 2 µg RNA (minimum RNA concentration 0.1 µg/µl) and mix by vortexing for 10 s. The final volume should be 100 µl.**

   For example, if the RNA concentration is 0.5 µg/µl, dilute 4 µl Enhancer R in 92 µl Buffer EC, and then add 4 µl RNA solution.

   IMPORTANT:

- Always mix Enhancer R with Buffer EC before adding RNA.
- Always keep the ratio of RNA to Enhancer R constant.

   Note: The best results are achieved when RNA of the highest purity is used for transfection. RNA purified with RNeasy Kits and Oligotex mRNA Kits is highly recommended.

4. **Incubate at room temperature (15–25°C) for 5 min, and then spin down the mixture for a few seconds to collect drops from the top of the tube.**
5. **Add 8 µl TransMessenger Transfection Reagent to the RNA–Enhancer R mixture. Mix by pipetting up and down 5 times, or by vortexing for 10 s.**
6. **Incubate the samples for 10 min at room temperature to allow transfection-complex formation.**
7. **While complex formation takes place, gently aspirate the growth medium from the plate, and carefully wash cells once with sterile PBS using 1.5–2 times the volume of medium used for cell seeding.**

   IMPORTANT: Do not allow the cells to become dry. Minimize the time they are without medium.

8. **Add 900 µl cell growth medium without serum or antibiotics to the tube containing the transfection complexes. Mix by pipetting up and down twice, and then immediately add the transfection complexes drop-wise onto the cells. Gently swirl the plate to ensure uniform distribution of the transfection complexes.**

   IMPORTANT: Use medium without serum or antibiotics to avoid RNase contamination.

9. **Incubate cells with the transfection complexes for 3 h under their normal growth conditions.**

   Note: If no cytotoxic effects are observed, the incubation time can be increased up to 4 h.

10. **Remove the complexes from the cells, wash cells once with PBS, and then add 2 ml fresh medium containing serum and antibiotics to the cells.**

11. Incubate cells under their normal growth conditions to allow protein expression.

Incubation time is determined by the assay and RNA used.

Note: Compared to DNA transfection, optimal expression levels may be obtained earlier following transfection with RNA. Cells transfected with a chloramphenicol acetyltransferase (CAT) reporter RNA are typically incubated for approximately 24 h post-transfection to obtain maximal levels of protein expression.

Table 4. Starting points for optimizing the transfection of adherent cells in different formats

Culture format	RNA, µg	Enhancer R, µl	Final volume of RNA–Enhancer R in Buffer EC, µl	TransMessenger Transfection Reagent, µl	Volume of medium added to complexes, µl*
Protocol step	**3**	**3**	**3**	**5**	**8**
96-well plate	0.25	0.5	15	1.5†	25
48-well plate	0.5	1.0	30	2.5†	50
24-well plate	0.8	1.6	100	4	100
12-well plate	1.6	3.2	100	6	300
6-well plate	2.0	4.0	100	8	900

* We recommend using medium without serum or antibiotics to avoid possible RNase contamination.

† If transfections are performed in 96- or 48-well plates, dilute TransMessenger Transfection Reagent with Buffer EC to a total volume of 10 µl or 20 µl, respectively, before addition to the RNA–Enhancer R mixture prepared in step 3.

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	
a) Sub-optimal efficiency	If the ratio of TransMessenger Transfection Reagent to RNA is sub-optimal, the overall TransMessenger Transfection Reagent:RNA ratio charge of the complexes may be negative, neutral, or strongly positive, which can lead to inefficient adsorption to the cell surface. Optimize the TransMessenger Transfection Reagent to RNA ratio according to the optimization guide (page 10).
b) Insufficient TransMessenger–RNA complex	If the transfection efficiency is lower than expected and cytoxicity is acceptably low, increase the overall amount of TransMessenger–RNA complex added to the cells. Refer to the pipetting scheme in Table 2 on page 12.
c) Sub-optimal incubation time for protein expression	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 type–RNA combination, a time course experiment may be necessary.
d) Influence of the RNA	Factors such as the size of the RNA and special features contained within it (e.g., IRES, cap, or poly-A tail) can influence its stability and/or the expression rate. Some RNA features may not be compatible with every cell type.

	Comments and suggestions
e) Sub-optimal cell density	If cell density at the time of TransMessenger–RNA complex addition is not at an optimal level, cells may not be in the optimal growth phase. This can lead to insufficient uptake of complexes into the cells or inefficient processing of the RNA of interest. For adherent cells, the optimal confluency for transfection is 80–90% (for single-stranded RNA) or 50–80% (for siRNA). Be sure to seed cells a minimum of 24 h prior to transfection.
f) Cell type–RNA combination	RNA species with features such as a cap, poly-A tail, or IRES will not necessarily be processed in every cell type. For example, RNA with an IRES may not be translated in COS-7 and HeLa cells. If no expression is observed change the cell type or, if possible, the RNA.
g) Poor RNA quality	RNA used for transfection should be of high quality. Impurities present in RNA preparations can potentially lower transfection efficiency. Degradation of RNA leads to decreased expression levels. RNA should be checked for degradation before transfection. We recommend purifying RNA using RNeasy Kits or Oligotex mRNA Kits.
h) Reporter assay problem	Include positive controls to ensure that the reporter assay is working properly.
i) Serum	Serum may contain RNases. We therefore recommend transfection in the absence of serum.
Excessive cell death	
a) Excessive exposure of cells to TransMessenger–RNA complexes	If excessive cell death is observed after 3 h incubation, reduce the time of cell–complex incubation to 1–2 h. In addition, wash particularly sensitive cells (e.g., primary cells) with 1.5–2 volumes of medium without serum instead of PBS after removal of the TransMessenger–RNA complexes.

	Comments and suggestions
b) Concentration of TransMessenger–RNA complexes is too high	If cell death is still excessive after reducing the exposure time, decrease the amount of TransMessenger–RNA complexes added to cells.
c) Cells are stressed	Avoid stressing cells with temperature shifts and long periods without medium during washing steps. It is particularly important for RNA transfection that the cells are in good condition. Therefore, ensure that cell density is not too low at transfection. For adherent cells, the optimal confluency for transfection is 80–90% (for single-stranded RNA) or 50–80% (for siRNA). Be sure to seed cells a minimum of 24 h before transfection.
d) Poor RNA quality	RNA used for transfection should be of high quality. Impurities present in RNA preparations can potentially lower transfection efficiency. Degradation of RNA leads to decreased expression levels. RNA should be checked for degradation before transfection. We recommend purifying RNA using RNeasy Kits or Oligotex mRNA Kits.
e) RNA-related effects	Toxic effects may arise if RNA encoding a toxic protein is transfected, or if too much RNA is used. In contrast, if insufficient RNA with a low translation rate is used, transfection efficiency may be too low. Optimize the amount of RNA according to the optimization guide (page 10) for each new RNA and/or cell type used.
f) Key gene is silenced	If the gene targeted in an RNAi experiment is key to the survival of the cell, silencing this gene will lead to cell death.
Variable transfection efficiencies in replicate experiments	
a) Inconsistent cell confluencies in replicate experiments	Count cells prior to seeding to ensure that the same number of cells is seeded for each experiment. Keep incubation times between seeding and complex addition consistent between experiments. Cells should be seeded no later than 24 h before transfection.

	Comments and suggestions
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 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 a low passage number (<50 splitting cycles).

No or very small gene silencing effect observed after siRNA transfection

a) Design of siRNA sub-optimal	The design of an siRNA can have a large effect on its gene silencing efficiency. QIAGEN offers expert advice on siRNA design for maximum gene silencing effect. For more information visit www.qiagen.com/products/genesilencing/librarysirna/sirnasets/sets.aspx.
b) Incubation time post-transfection too short	The gene silencing effect observed on the protein level is dependent on a protein's expression level and its rate of turnover within the cell. Perform a time course experiment to determine the optimal time point for analysis.
c) Problems with experimental design	RNAi effects may not be seen for some genes targeted with certain siRNAs in some cell types. If possible, repeat experiments using a different cell type and/or siRNA. Include where possible both positive and negative controls in your experiments. QIAGEN offers siRNA that has been functionally tested for specific gene silencing (see ordering information).

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.
TransMessenger Transfection Reagent (0.5)	For 60 transfections in 6-well plates or 80 transfections in 12-well plates	301525
Related products		
RNeasy Mini Kits — for purification of total RNA from cells, tissues, and yeast		
RNeasy Mini Kit (50)*	50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74104
RNeasy Midi Kit (10)*	10 RNeasy Midi Spin Columns, Collection Tubes (15 ml), RNase-free Reagents and Buffers	75142
RNeasy Maxi Kit (12)	12 RNeasy Maxi Spin Columns, Collection Tubes (50 ml), RNase-free Reagents and Buffers	75162
Oligotex mRNA Mini Kit† — for mRNA purification from total RNA and for in vitro transcript cleanup		
Oligotex mRNA Mini Kit (12)	For 12 mRNA minipreps: 200 µl Oligotex Suspension, Small Spin Columns, Collection Tubes (1.5 ml), RNase-Free Reagents and Buffers	70022
Custom siRNA oligonucleotides‡		
FlexiTube	siRNA 20 nmol, 5 nmol, or 1 nmol siRNA delivered in tubes	Varies
FlexiTube GeneSolution	4 siRNAs (1 nmol) recommended for a gene; delivered in tubes	Varies
FlexiPlate siRNA	siRNA in 96-well and 384-well plates; minimum order 36 siRNAs	Varies

* Other kit sizes are available; see www.qiagen.com.

† Not available in Japan.

‡ Numerous 3' and 5' modifications are available on request. For an up-to-date list, and information on how to order custom siRNA, please visit www.qiagen.com/sirna.

Product	Contents	Cat. no.
HP Custom siRNA	siRNA purified to >90% (20 nmol), modifications and labels available	Varies
QIAGEN AllStars RNAi Controls	Positive, negative, transfection, downstream, reporter, and interferon controls	Varies
AllStars Hs Cell Death siRNA (5 nmol)*	Positive cell death phenotype control	1027298
AllStars Negative Control siRNA (5 nmol)*	Negative control to indicate if changes in phenotype or gene expression are nonspecific	1027280
QuantiFast SYBR Green RT-PCR Kit — for fast, one-step qRT-PCR using SYBR Green I for gene expression analysis		
QuantiFast SYBR Green RT-PCR Kit (400)*	For 400 x 25 µl reactions: 3 x 1.7 ml 2x QuantiFast SYBR Green RT-PCR Master Mix (contains ROX™ dye), 100 µl QuantiFast RT Mix, 2 x 2 ml RNase-Free Water	204154
QuantiFast Probe RT-PCR Kit — for fast, one-step qRT-PCR using sequence-specific probes for gene expression analysis		
QuantiFast Probe RT-PCR Kit (400)*†	For 400 x 25 µl reactions: 3 x 1.7 ml 2x QuantiFast Probe RT-PCR Master Mix (contains ROX dye), 100 µl QuantiFast RT Mix, 2 x 2 ml RNase-Free Water	204454

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

* Other kit sizes are available; see www.qiagen.com.

† Kit format without ROX in the master mix is also available; see www.qiagen.com.

Notes

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1073577 09/2012

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