Third Edition

May 2009

RNAi Human/Mouse Starter Kit Handbook

For RNAi in human and mouse cells

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Contents

Kit Contents

RNAi Human/Mouse Starter Kit	
Catalog no.	**301799**
Transfections per kit	**>160 transfections**
HiPerFect Transfection Reagent	0.5 ml
AllStars Negative Control siRNA	5 nmol
AllStars Hs Cell Death Control siRNA	5 nmol
Hs/Mm_MAPK1 Control siRNA	5 nmol
Sterile, RNase-Free Water	1 ml
Handbook	1

Shipping and Storage

HiPerFect Transfection Reagent is supplied as a ready-to-use solution and is shipped at room temperature (15–25°C) without loss of stability. However, it should be stored at 2–8°C upon arrival. HiPerFect Transfection Reagent does not need to be stored on ice during the transfection procedure.

siRNAs are shipped at room temperature and should be stored at –20°C upon arrival. The siRNAs should therefore be stored separately from the rest of the kit.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of RNAi Human/Mouse Starter Kit is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

The RNAi Human/Mouse Starter Kit is intended for molecular biology applications. It is neither intended for the diagnosis, prevention, or treatment of a disease, nor has it been validated for such use either alone or in combination with other products. Therefore, the performance characteristics of this product for clinical use, (i.e., diagnostic, prognostic, therapeutic, or blood banking), is unknown.

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.

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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At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the RNAi Human/Mouse Starter Kit or QIAGEN products in general, please do not hesitate to contact us.

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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

Introduction

The RNAi Human/Mouse Starter Kit allows easy establishment and optimization of gene silencing in a human or mouse cell line of interest. The kit contains positive and negative control siRNAs, HiPerFect Transfection Reagent, and protocols intended as starting points for RNAi experiments.

Principle and procedure

RNAi with low siRNA concentrations using HiPerFect Transfection Reagent

HiPerFect Transfection Reagent has been developed for highly efficient siRNA transfection and gene knockdown in eukaryotic cells using low siRNA concentrations. Research suggests that off-target effects, which may produce misleading results in RNAi experiments, can be largely avoided by using low siRNA concentrations (1, 2).

Off-target effects occur when siRNAs affect the expression of nonhomologous or partially homologous gene targets. These effects can include mRNA degradation, inhibition of translation, or induction of a nonspecific interferon response (3–6). The mechanisms of off-target effects are not fully understood. They may be caused by siRNA targeting mRNA with close homology to the target mRNA, by siRNAs functioning like miRNAs, or by a cellular response to siRNA toxicity.

HiPerFect Transfection Reagent is a unique blend of cationic and neutral lipids that enables effective siRNA uptake and high gene knockdown with low siRNA concentrations, minimizing the risk of off-target effects. HiPerFect Transfection Reagent is provided as a ready-to-use solution — just add the reagent to your diluted siRNA, mix, incubate, and add the complexes to the cells. Transfections can be performed in the presence of serum, eliminating the need to remove complexes from the cells.

Positive control siRNA targeted against MAPK1

The RNAi Human/Mouse Starter Kit includes positive control siRNA targeted against the protein kinase MAPK1 (also called MAPK2 and Erk2). MAPK1 is ubiquitously expressed in both human and mouse cell lines, making it a suitable target gene for control experiments. The sequence of the Hs/Mm_MAPK1 Control siRNA provided in this kit is homologous to both the human and mouse MAPK1 mRNA sequences, (GenBank® accession number NM_002745 [human] and NM_011949 [mouse]). Western blot and quantitative, real-time RT-PCR analysis of mouse and human cell lines after transfection with the Hs/Mm_MAPK1 Control siRNA showed highly efficient gene silencing. As the Hs/Mm_MAPK1 Control siRNA provides high levels of gene knockdown, it can be used to optimize experimental conditions, and it can be routinely transfected in parallel with the siRNA under study.

Human and mouse MAPK1 can be easily detected by western blotting using the MAPK1-specific Tag·100 antibody (cat. no. 34680). The Tag·100 antibody was originally developed for protein detection when using QIAGEN's pQE-100 DoubleTag Vector for protein expression. However, it is also effective for monitoring gene silencing of human and mouse MAPK1 at the protein level. Alternatively, QuantiTect® Primer Assays for MAPK1 are validated primer sets that can be used for quantitative, real-time RT-PCR with SYBR® Green based detection. The Hs_MAPK1_1_SG QuantiTect Primer Assay (cat. no. QT00065933) and the Mm_Mapk1_1_SG QuantiTect Primer Assay (cat. no. QT00133840) are designed for human and mouse, respectively.

For gene silencing experiments using rat cells, we recommend purchase of Rn_Mapk1 Control siRNA (cat. no. 1027277). This siRNA has been shown to provide >70% knockdown of the rat MAPK1 gene. For downstream analysis of rat MAPK1 knockdown, the Rn_Mapk1_1_SG QuantiTect Primer Assay (cat. no. QT00190379) can be used for SYBR Green based, quantitative, real-time RT-PCR and the Tag·100 antibody can be used for western blotting.

Positive control siRNA induces cell-death phenotype

AllStars Hs Cell Death Control siRNA is a blend of highly potent siRNAs targeting ubiquitously expressed human genes that are indispensable for cell survival. Knockdown of these genes induces a high degree of cell death which is visible by light microscopy. AllStars Hs Cell Death Control siRNA is a useful tool for siRNA transfection optimization and for routine use as a positive control in human cells. For details on monitoring transfection efficiency using AllStars Hs Cell Death Control siRNA, see page 20.

Highly validated, negative control siRNA

Negative, nonsilencing controls, consisting of siRNAs that have no known homology to mammalian genes, are used to control for nonspecific silencing effects. If altered expression or phenotype are observed in cells transfected with negative control siRNA, these changes are nonspecific (i.e., due to transfection procedures or siRNA toxicity and not sequence complementarity). Nonspecific effects should be minimal to ensure reliable RNAi results.

AllStars Negative Control siRNA, included in this kit, is the most thoroughly tested and validated negative control siRNA currently available. This siRNA has no homology to any known mammalian gene. It has been validated using Affymetrix® GeneChip® arrays and a variety of cell-based assays and shown to exhibit minimal nonspecific effects on gene expression and phenotype. Cloning experiments confirmed that AllStars Negative Control siRNA enters RISC. AllStars Negative Control siRNA is patent-pending and the sequence is proprietary. For more details on the range of validation tests performed on AllStars Negative Control siRNA, visit www.qiagen.com/AllStars.

Rapid transfection using the Fast-Forward Protocol

This handbook contains 2 protocols for siRNA transfection of adherent cells in 24-well plates, the Traditional Protocol and the Fast-Forward Protocol. The Traditional Protocol describes the commonly used transfection procedure of seeding the cells 24 hours before the day of transfection (see page 24). The Fast-Forward Protocol allows cell seeding and transfection on the same day and is particularly useful for experiments where speed is important (see page 22). For high-throughput applications, protocols for reverse transfection in 96-well and 384-well plates are also included in the handbook (page 26 and page 28).

For large-scale transfection, a protocol is provided for transfection in 100 mm dishes on page 30. A protocol for long-term gene silencing is included on page 32. This protocol is for the study of phenotypic effects that require knockdown for periods longer than 3–4 days before they can be observed. Gene knockdown using siRNA is transient and expression usually increases again several days after transfection, especially when the cells are subcultured. However, the long-term gene silencing protocol allows gene knockdown for over 2 weeks. The protocol uses HiPerFect Transfection Reagent to deliver siRNA every time the cells are diluted and replated. This is possible because transfection with HiPerFect Reagent ensures extremely low levels of cytotoxicity, so cells remain viable and healthy after multiple cycles of dilution and transfection.

Transfection of suspension cells

Protocols for transfection of suspension cells, macrophages, and differentiated macrophages are provided in the *HiPerFect Transfection Reagent Handbook* (www.qiagen.com/HB/HiPerFect).

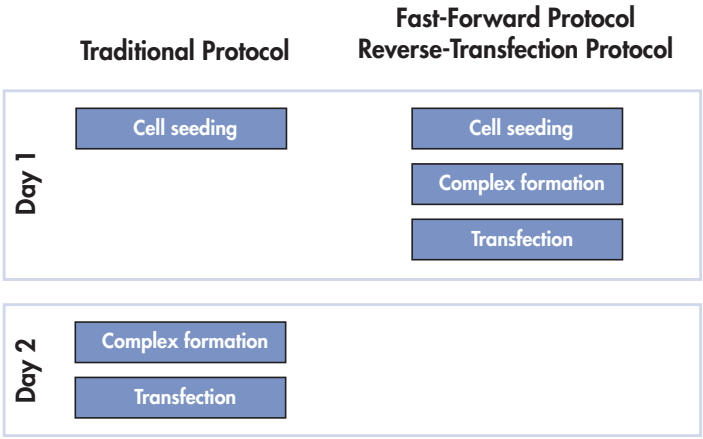


Figure 1. The Fast-Forward Protocol saves time and labor. In the Fast-Forward Protocol (and Reverse-Transfection Protocols), cell seeding, complex formation, and transfection are all performed on the same day. In the Traditional Protocol, cell seeding is performed the day before transfection.

RNA interference

RNA interference (RNAi) is a biological process in which the introduction of double-stranded RNA (dsRNA) into a cell results in targeted posttranscriptional gene silencing. Historically, RNAi has been used as a tool for functional genomics research in *Caenorhabditis elegans* and Drosophila. Initial attempts to activate the RNAi pathway in mammalian cells were unsuccessful, since the introduction of dsRNA >30 nucleotides (nt) in length leads to nonspecific suppression of gene expression. However, as RNAi became better understood, scientists discovered that double-stranded short interfering RNA (siRNA) oligos of 23 nt could be used to mediate gene silencing in mammalian cells. The application of RNAi to mammalian cells is revolutionizing the field of functional genomics. In 2006, the huge contribution of the discoverers of RNAi, Andrew Z. Fire and Craig C. Mello, was recognized when they were awarded the Nobel Prize in Medicine.

Discovery of RNAi

The origins of RNAi involved a number of scientists working in different research fields, who observed a phenomenon that they did not immediately understand. Plant biologists attempting to boost the activity of the gene for chalcone synthase in petunias by introducing a powerful promoter sequence into a transgene observed that instead of the deep purple color they expected, flowers were variegated, or completely white. The researchers concluded that the introduced chalcone synthase transgene had somehow suppressed both itself and the endogenous petunia gene, and so named this phenomenon cosuppression (7).

Another research group working on genes expressed from the potato virus X in tobacco plants hoped that viral proteins produced by the plants would stimulate a defense mechanism, allowing the plants to resist subsequent attack by the virus. To their surprise, the plants with the strongest resistance to the virus were those in which the introduced gene was silent. The researchers concluded that the introduced gene was suppressing expression both of itself and of the same gene in the virus (8).

In fungi, gene silencing was observed during attempts to boost the production of an orange pigment by the mold *Neurospora crassa*. Extra copies of a gene involved in making a carotenoid pigment were introduced into mold cells. However, rather than turning a deeper orange, a third of the engineered mold appeared yellow or white. Something had suppressed the pigment genes. The observed phenomenon was named 'quelling' (9, 10).

Other scientists working with *C. elegans* obtained unexpected results in their antisense RNA experiments. The antisense approach to gene silencing involves injecting an organism with RNA sequence complementary to mRNA transcribed from a target gene. The antisense RNA and sense mRNA hybridize and block production of the encoded protein. However in one case, a sense strand, injected as a control, led to gene silencing (11). This effect was later explained by the presence in the RNA preparation of very small amounts of the corresponding antisense strand. The presence of dsRNA led to what we now recognize as an RNAi effect (12). Antisense experimental theory had incorrectly predicted that these small contaminants would have no effect on gene expression.

Using *C. elegans,* Fire, Mello, and coworkers demonstrated that injection of dsRNA was more effective in gene silencing than injection of sense or antisense strands alone (13). Only a few molecules of injected dsRNA were required to shut down expression of protein in a cell. The dsRNA gene-silencing mechanism was found to be highly gene-specific and to be part of a complex biological regulation system. The phenomenon of gene silencing using dsRNA was termed "RNA interference" (13).

RNAi in mammalian cells

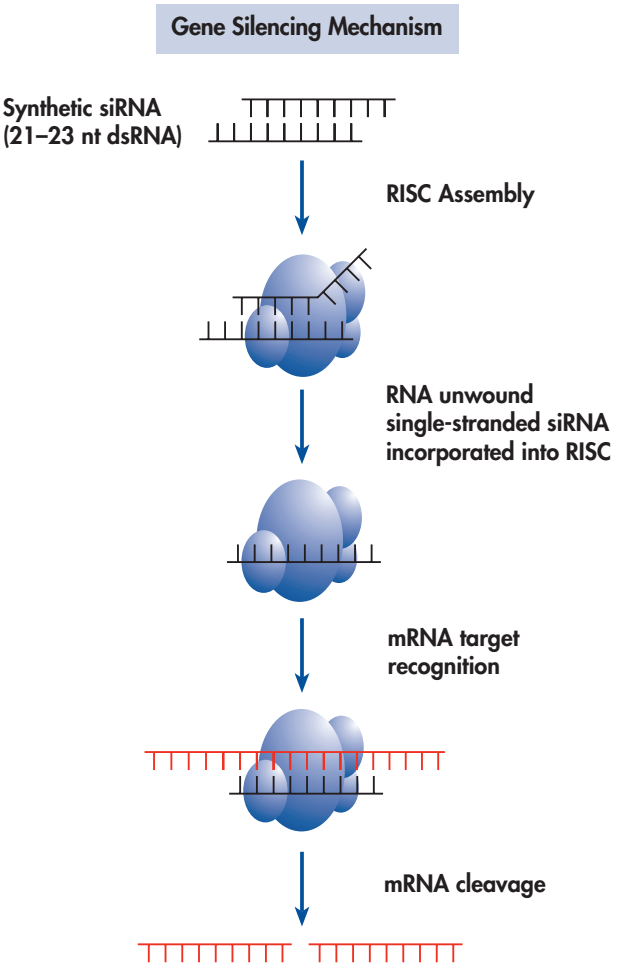
Initial attempts to activate the RNAi pathway in mammalian cells were unsuccessful, since the introduction of dsRNA leads to activation of the dsRNA-dependent protein kinase PKR and 2',5'-oligoadenylate synthetase (2',5'-AS). The activation of these two enzymes triggers a nonspecific shutdown of protein synthesis and nonspecific degradation of mRNA. Consequently, some researchers thought that gene-specific RNAi was not possible in mammalian systems.

Elbashir et al. showed that 21–23 nt dsRNA fragments successfully trigger RNAi in an in vitro system using Drosophila lysate. They also demonstrated that chemically synthesized 21 nt siRNA duplexes specifically suppress the expression of endogenous and heterologous genes in different mammalian cell lines, including human 293 and HeLa cells (14). A key discovery from these studies was that no nonspecific gene silencing effects were seen in mammalian cells by transfection of short dsRNA sequences (<30 nt). These results showed that 21 nt siRNA duplexes can be used as a new tool for studying gene function in mammalian cells and may eventually be used as gene-specific therapeutics.

Work by Caplen et al. (15) confirmed and extended the reports of siRNA-mediated RNAi in mammalian cell extracts. They demonstrated that identically sized synthetic siRNAs can induce gene-specific inhibition of expression in *C. elegans*, human, and mouse cells. Consistent with this hypothesis, numerous studies have since shown that dsRNA-induced gene silencing occurs in a number of different eukaryotic species (13, 17–27). The finding that the size of functional dsRNA fragments is conserved in plants and animals suggests a highly conserved mechanism in nature (16).

How does RNAi lead to gene silencing?

The basic mechanism of RNAi is a multi-step process (see flowchart). In cultured mammalian cells, RNAi is mediated by 21nt RNA duplexes with symmetric 2-nt 3' overhangs. These siRNAs are introduced into a cell by transfection and lead to degradation of mRNA having the same sequence, thereby silencing gene expression.



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
- siRNA of interest. siRNA for every human, mouse, and rat gene is available at the GeneGlobe® Web portal (www.qiagen.com/GeneGlobe).
- Tools for monitoring gene silencing at the mRNA or protein level. QIAGEN provides MAPK1-specific Tag·100 Antibody (cat. no. 34680) for western blot analysis of the MAPK1 protein. For quantitative, real-time RT-PCR analysis, QuantiTect Primer Assays are ready-to-use primer sets for quantitative, real-time RT-PCR using SYBR Green detection. The Hs_MAPK1_1_SG QuantiTect Primer Assay (cat. no. QT00065933) and the Mm_Mapk1_1_SG QuantiTect Primer Assay (cat. no. QT00133840) are designed for human and mouse, respectively. For more information, visit www.qiagen.com/goto/assays.
- For RNAi in rat cells: we recommend use of Rn_Mapk1 Control siRNA (cat. no. 1027277) as a positive control. For quantitative, real-time RT-PCR analysis, the Rn_Mapk1_1_SG QuantiTect Primer Assay (cat. no. QT00190379) is a ready-to-use primer set for quantitative, real-time RT-PCR using SYBR Green detection. The Tag·100 Antibody can be used for western blotting.
- For long-term gene silencing: Sterile PBS and trypsin/EDTA (see protocol on page 32 for details)

Important Notes

Choosing a target sequence and designing an siRNA

Predesigned siRNA that has been designed using innovative HP OnGuard siRNA Design is available for every human, mouse, and rat gene at www.qiagen.com/GeneGlobe. For cost-effective RNAi, FlexiTube siRNAs are available in 1-nmol amounts. FlexiTube GeneSolution provides 4 siRNAs for each target gene. For 96-well plate formats, FlexiPlate siRNA enables complete flexibility to choose siRNAs, plate layout, and amounts.

Quality of siRNA

Optimal results are achieved when high-purity siRNA is used for transfection. QIAGEN HPP Grade siRNA (>90% pure) is well suited for the transfection of many cell lines. FlexiTube siRNAs and FlexiTube GeneSolutions are HPP Grade siRNA. For further information on QIAGEN siRNA, visit www.qiagen.com/siRNA.

Calculating siRNA concentrations

Approximate values for a double-stranded, 21 nt siRNA molecule:

- 20 µM siRNA is equivalent to approximately 0.25 µg/µl
- Molecular weight of a 21 nt siRNA is approximately 13–15 µg/nmol (sequence-dependent)

Optimizing siRNA transfection

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

Amount of siRNA

The amount of siRNA used is critical for efficient transfection and gene silencing. The recommended starting concentration for transfection of siRNA is 5 nM. A pipetting scheme for optimizing siRNA transfection of adherent cells in 24-well plates is shown in Table 1 (next page).

Ratio of HiPerFect Transfection Reagent to siRNA

The ratio of HiPerFect Transfection Reagent to siRNA should be optimized for every new cell type and siRNA combination used. As a starting point for optimization, we recommend 5 nM siRNA and 3 µl HiPerFect Transfection Reagent when using 24-well plates. To optimize siRNA transfection in 24-well plates, prepare separate transfection mixtures according to Table 1 (next page).

Please note that Table 1 is intended only as a guideline for starting amounts of siRNA and reagent. These amounts worked well as a starting point for transfection optimization in the range of cell lines that have been tested using HiPerFect Transfection Reagent. If necessary, it is also possible to further reduce the volume of HiPerFect Transfection Reagent without significantly compromising performance.

Table 1. Pipetting scheme for optimizing transfection of adherent cells in 24-well plates*

Amount (conc.) of siRNA	75 ng (10 nM)	75 ng (10 nM)	75 ng (10 nM)
Volume of HiPerFect Reagent	1.5 µl	3 µl	4.5 µl
Amount (conc.) of siRNA	37.5 ng (5 nM)	**37.5 ng (5 nM)**	37.5 ng (5 nM)
Volume of HiPerFect Reagent	1.5 µl	**3 µl**	4.5 µl
Amount (conc.) of siRNA	7.5 ng (1 nM)	7.5 ng (1 nM)	7.5 ng (1 nM)
Volume of HiPerFect Reagent	1.5 µl	3 µl	4.5 µl

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

Cell density at transfection

The optimal confluency for transfection of adherent cells with siRNA is 50–80% when using the Traditional Protocol (seeding the day before transfection). 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 before seeding and by keeping the interval between seeding and transfection constant. This ensures that the cell density is not too high and that the cells are in optimal physiological condition at transfection. The recommended number of cells to seed for different formats using the Fast-Forward Protocol (seeding and transfection on the same day) or the Traditional Protocol (seeding the day before transfection) is shown in Table 2 (next page). An up-to-date list of cell types successfully transfected using HiPerFect Transfection Reagent is available at the Transfection Cell Database. In addition, transfection protocols for various cell types and formats are available from the TransFect Protocol Database. Both databases can be accessed at www.qiagen.com/TransfectionTools.

Table 2. Recommended number of cells to seed for different formats

Culture format	Suggested number of adherent cells to seed	
	Fast-Forward Protocol	Traditional Protocol
96-well plate	1–5 x 10^4	0.5–3 x 10^4
48-well plate	2–8 x 10^4	1–4 x 10^4
24-well plate	0.4–1.6 x 10^5	2–8 x 10^4
12-well plate	0.8–3 x 10^5	0.4–1.6 x 10^5
6-well plate	1.5–6 x 10^5	0.8–3 x 10^5
60 mm dish	0.3–1.2 x 10^6	1.5–6 x 10^5

High knockdown and minimal risk of off-target effects

HiPerFect Transfection Reagent enables highly effective siRNA uptake, allowing gene silencing using low siRNA concentrations without compromising knockdown efficiency. In the data shown (Figure 2), efficient knockdown (>80%) is achieved with siRNA concentrations in the range of 1–50 nM. Transfection of 1 nM siRNA resulted in 86% knockdown and transfection of 5 nM siRNA increased the knockdown efficiency to 96%. Depending on the purpose of the RNAi experiment, the optimal concentration of siRNA to use may be 1 nM (minimal risk of off-target effects and efficient knockdown) or 5 nM (higher knockdown efficiency). To achieve the highest possible knockdown levels, it may be necessary to increase siRNA concentrations above the levels indicated in the optimization scheme in Table 1. The optimal siRNA concentration will also depend on siRNA potency, cell type, and the target gene.

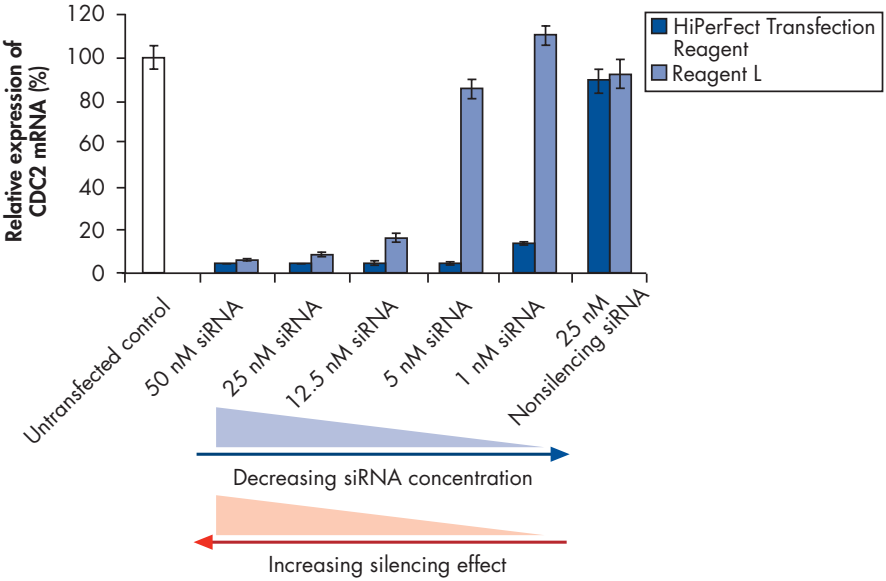


Figure 2. HiPerFect Reagent provides effective CDC2 knockdown even at low siRNA concentrations. HeLa S3 cells were transfected with a range of concentrations of siRNA targeted against CDC2 using HiPerFect Transfection Reagent from QIAGEN or Reagent L from another supplier. Nonsilencing siRNA was also transfected. After 48 hours, cells were harvested and total cellular RNA was purified using the RNeasy® system, and reverse transcribed using the Omniscript® RT Kit. The resultant cDNA was used for quantitative, real-time RT-PCR. Expression of CDC2 was normalized to expression of GAPDH. Values derived from real-time RT-PCR of control, untransfected cells were set at 100% and the relative expression levels of cells transfected with the experimental siRNA are shown.

Optimizing siRNA transfection in different formats

Table 3 gives starting points for optimization of siRNA transfection in different plate and dish formats for both the Fast-Forward Protocol and the Traditional Protocol. Table 4 shows the corresponding volumes of siRNA stock to use for each siRNA amount.

Table 3. Starting points for optimizing transfection of adherent cells in different formats.

Culture format	Volume of medium on cells (µl)	siRNA amount (ng)	Final volume of diluted siRNA (µl)	Volume of HiPerFect Reagent (µl)	Final siRNA conc. (nM)
384-well plate	40	3.125	1–3	0.5*	5
96-well plate	175	12.5	1–3	0.75†	5
48-well plate	250	19	50	1.5	5
24-well plate	500	37.5	100	3	5
12-well plate	1100	75	100	6	5
6-well plate	2300	150	100	12	5
60 mm dish	4000	256	100	20	5
100 mm dish	7000	600	1000	40	5

* If transfections are performed in 384-well plates, dilute 0.5 µl HiPerFect Transfection Reagent with medium without serum to a total volume of 10 µl before adding to the diluted siRNA prespotted in protocol step 1.

† If transfections are performed in 96-well plates, dilute 0.75 µl HiPerFect Transfection Reagent with medium without serum to a total volume of 25 µl before adding to the diluted siRNA prespotted in protocol step 1.

Table 4. Volumes of siRNA stock for different siRNA amounts

Culture format	siRNA amount (ng)	Equivalent volume of 0.2 µM siRNA stock (µl)*	Equivalent volume of 2 µM siRNA stock (µl)*	Equivalent volume of 20 µM siRNA stock (µl)*
384-well plate	3.125	1.25	–	–
96-well plate	12.5	5	–	–
48-well plate	19	7.5	–	–
24-well plate	37.5	–	1.5	–
12-well plate	75	–	3	–
6-well plate	150	–	6	–
60 mm dish	256	–	–	1
100 mm dish	600	–	–	2.4

* To make a 0.2 µM siRNA stock, dilute 10 µl of a 20 µM stock to a final volume of 1000 µl. To make a 2 µM siRNA stock, dilute 10 µl of a 20 µM stock to a final volume of 100 µl. See "Important points before starting" at the beginning of each protocol for details of how to make a 20 µM stock solution using siRNA provided in this kit.

Transfection in multiwell plates — preparing a master mix

If you are performing transfection in multiwell plates, prepare a master mix of transfection complexes or of transfection reagent and culture medium (depending on the protocol) for distribution into plate wells.

- Calculate the required volumes of each component and the total volume before you prepare the master mix.
- Prepare 10% more master mix than is required to allow for pipetting errors (i.e., for a 48-well plate, prepare enough master mix for 53 wells).
- Add and mix the components of the master mix according to the instructions in the protocol.
- Use a repeat pipet to distribute the master mix.

Monitoring transfection efficiency using AllStars Hs Cell Death Control siRNA

AllStars Hs Cell Death Control siRNA is a blend of siRNAs targeting human genes that are essential for cell survival. Successful transfection of AllStars Hs Cell Death Control siRNA leads to a high degree of cell death. This allows the use of AllStars Hs Cell Death Control siRNA as phenotypic control.

When establishing RNAi in start-up experiments or in a new cell line, it is necessary to perform multiple transfections under different conditions to determine the optimal conditions for maximum transfection efficiency. These experiments can be performed by transfecting AllStars Hs Cell Death Control siRNA. Observation of cells by light microscopy should be performed 72 hours after transfection to allow sufficient time for the development of the phenotype. Transfection conditions that result in the greatest degree of cell death in comparison to transfection with a nonsilencing control siRNA, such as AllStars Negative Control siRNA provided in this kit, can be maintained in future experiments. Comparison to AllStars Negative Control siRNA is recommended to rule out the presence of additional nonspecific, off-target cytotoxic effects.

Monitoring gene silencing at the protein level

Gene silencing can be monitored at either the protein or the mRNA level. Protein expression analysis is by western blotting, immunofluorescence, or FACS® analysis. A mouse anti-Tag·100 antibody that recognizes endogenous mammalian MAPK1 is available from QIAGEN (cat. no. 34680). This antibody can be used to monitor MAPK1 gene silencing by western blot analysis. A general protocol for western blotting is given in the Appendix, page 37.

Monitoring gene silencing at the mRNA level

Silencing is usually monitored at the mRNA level by real-time RT-PCR, microarray analysis, or northern blotting. Information about working with RNA and a northern blotting protocol are available at www.qiagen.com/literature/BenchGuide. The RNeasy system is recommended for purification of high-quality RNA. Alternatively, the FastLane Cell cDNA Kit allows high-speed preparation of cDNA without RNA purification.

The RNAi Human/Mouse Starter Kit can be used in combination with QuantiTect Primer Assays and QuantiTect or QuantiFast® SYBR Green Kits for downstream analysis. The Hs_MAPK1_1_SG QuantiTect Primer Assay or the Mm_Mapk1_1_SG QuantiTect Primer Assay, for human and mouse respectively, can be used together with the QuantiTect and QuantiFast SYBR Green RT-PCR or PCR Kits for two-step or one-step quantitative, real-time RT-PCR. For reverse transcription in two-step RT-PCR, the QuantiTect Reverse Transcription Kit allows rapid cDNA synthesis and includes an integrated genomic DNA removal step. For high-throughput analysis, the FastLane Cell SYBR Green Kit allows real-time, one-step RT-PCR direct from cell lysates. Further information on products for gene expression analysis is available at www.qiagen.com/goto/assays. All expression data should be compared with levels of a housekeeping gene, such as GAPDH, to normalize for variable amounts of RNA in different samples.

Protocol: Fast-Forward Transfection of Adherent Cells with siRNA

This protocol is for convenient and rapid transfection of adherent cells in a single well of a 24-well plate. Cell plating and transfection are performed on the same day. This protocol is provided as a starting point for optimization of siRNA transfection in mammalian cells using the RNAi Human/Mouse Starter Kit. For a few sensitive cell types, it may be necessary to use the Traditional Protocol, where cells are plated the day before transfection (page 24).

Important points before starting

- Be sure to work in an RNase-free environment.
- To prepare siRNAs provided in this kit: Add 250 µl RNase-free water (provided) to the lyophilized siRNA (5 nmol) to obtain a 20 µM solution.

  Perform your experiment or store at –20°C. Avoid repeated freeze-thaw cycles by aliquoting suitable amounts prior to storage.

Procedure

1. **Shortly before transfection, seed 0.4–1.6 x 10^5 cells per well of a 24-well plate in 0.5 ml of an appropriate growth medium containing serum and antibiotics.**
2. **For the short time until transfection, incubate the cells under normal growth conditions (typically 37°C and 5% CO_2).**

   Cells may alternatively be seeded after step 3 of this protocol.
3. **Dilute 37.5 ng siRNA in 100 µl culture medium without serum to give a final siRNA concentration of 5 nM. Add 3 µl of HiPerFect Transfection Reagent to the diluted siRNA and mix by vortexing.**

   IMPORTANT: The amount of HiPerFect Transfection Reagent and siRNA required for optimal performance may vary, depending on the cell line and gene target. For details on optimization of the siRNA to HiPerFect Transfection Reagent ratio, see page 15.
4. **Incubate the samples for 5–10 min at room temperature (15–25°C) to allow the formation of transfection complexes.**
5. **Add the complexes drop-wise onto the cells. Gently swirl the plate to ensure uniform distribution of the transfection complexes.**

6. **Incubate the cells with the transfection complexes under their normal growth conditions and monitor gene silencing after an appropriate time (e.g., 6–72 h after transfection, depending on experimental setup). Change the medium as required.**

   Note: The optimal incubation time for gene silencing analysis depends on the cell type, the gene targeted, and the method of analysis. This can be determined by performing a time-course experiment.

   Note: If monitoring transfection efficiency using AllStars Hs Cell Death Control siRNA, cells should be incubated for 72 hours prior to observation by light microscopy.

Protocol: Transfection of Adherent Cells with siRNA (Traditional Protocol)

This protocol is for transfection of adherent cells in a single well of a 24-well plate if seeding cells the day before transfection is preferred. It is provided as a starting point for optimization of siRNA transfection in mammalian cells using HiPerFect Transfection Reagent. For some cell types, such as HepG2, the Fast-Forward Protocol may give higher gene silencing effects at very low siRNA concentrations compared to this protocol (page 22).

Important points before starting

- Be sure to work in an RNase-free environment.
- To prepare siRNAs provided in this kit: Add 250 µl RNase-free water (provided) to the lyophilized siRNA (5 nmol) to obtain a 20 µM solution.

  Perform your experiment or store at –20°C. Avoid repeated freeze-thaw cycles by aliquoting suitable amounts prior to storage.

Procedure

1. **The day before transfection, seed 2–8 x 10^4 cells per well of a 24-well plate in 0.5 ml of an appropriate growth medium containing serum and antibiotics.**
2. **Incubate the cells under normal growth conditions (typically 37°C and 5% CO_2).**
3. **On the day of transfection, dilute 37.5 ng siRNA in 100 µl culture medium without serum to give a final siRNA concentration of 5 nM. Add 3 µl of HiPerFect Transfection Reagent to the diluted siRNA and mix by vortexing.**

   IMPORTANT: The amount of HiPerFect Transfection Reagent and siRNA required for optimal performance may vary, depending on the cell line and gene target. For details on optimization of the siRNA to HiPerFect Transfection Reagent ratio, see page 15.
4. **Incubate the samples for 5–10 min at room temperature (15–25°C) to allow the formation of transfection complexes.**
5. **Add the complexes drop-wise onto the cells. Gently swirl the plate to ensure uniform distribution of the transfection complexes.**

6. **Incubate the cells with the transfection complexes under their normal growth conditions and monitor gene silencing after an appropriate time (e.g., 6–72 h after transfection, depending on experimental setup). Change the medium as required.**

   Note: The optimal incubation time for gene silencing analysis depends on the cell type, the gene targeted, and the method of analysis. This can be determined by performing a time-course experiment.

   Note: If monitoring transfection efficiency using AllStars Hs Cell Death Control siRNA, cells should be incubated for 72 hours prior to observation by light microscopy.

Protocol: Reverse Transfection of Adherent Cells with siRNA in 96-Well Plates

This protocol is provided as a starting point for optimization of siRNA transfection of adherent cells in a single well of a 96-well plate using the RNAi Human/Mouse Starter Kit. Starting points for optimizing transfection in other formats are listed in Table 3 on page 19. In this protocol, cell plating and transfection are performed on the same day.

Important points before starting

- Cells should be in optimal physiological condition at the time of transfection. The optimal amount of cells seeded depends on the cell type and time of analysis.
- To prepare siRNAs provided in this kit: Add 250 µl RNase-free water (provided) to the lyophilized siRNA (5 nmol) to obtain a 20 µM solution.

  Perform your experiment or store at –20°C. Avoid repeated freeze-thaw cycles by aliquoting suitable amounts prior to storage.

Procedure

1. **Spot 12.5 ng siRNA in a volume of 1–3 µl into a single well of a 96-well plate (this will give a final siRNA concentration of 5 nM after addition of cells to complexes in step 4).**

   Note: After this step, siRNA may be stored at –20°C for long time periods. siRNA may be stored in solution or may be dried in the plate at room temperature (15–25°C) before storage.

   Note: If preferred, siRNA can be spotted in a volume of 25 µl into each well. In this case, 150 µl culture medium (containing 1–5 x 10^4 cells) should be added in step 4.

2. **Add 0.75 µl of HiPerFect Transfection Reagent to 24.25 µl of culture medium without serum. Add the diluted HiPerFect Transfection Reagent to the prespotted siRNA.**

   Note: To ensure accurate pipetting, diluted HiPerFect Reagent should be prepared in a larger volume for use in multiple wells (see "Transfection in multiwell plates — preparing a master mix" on page 20). Then add 25 µl of the dilution to a single well.

   IMPORTANT: The amount of HiPerFect Transfection Reagent and siRNA required for optimal performance may vary, depending on the cell line and gene target. For details on optimization of the siRNA to HiPerFect Transfection Reagent ratio, see page 15.

3. **Incubate for 5–10 min at room temperature (15–25°C) to allow formation of transfection complexes.**
4. **Seed 1–5 x 10^4 cells in 175 µl of an appropriate culture medium (containing serum and antibiotics) into the well, on top of the siRNA–HiPerFect Reagent transfection complexes.**
5. **Incubate the cells with the transfection complexes under their normal growth conditions and monitor gene silencing after an appropriate time (e.g., 6–72 h after transfection, depending on experimental setup). Change the medium as required.**

   Note: The optimal incubation time for gene silencing analysis depends on cell type, the gene targeted, and the method of analysis. This can be determined by performing a time-course experiment.

   Note: If monitoring transfection efficiency using AllStars Hs Cell Death Control siRNA, cells should be incubated for 72 hours prior to observation by light microscopy.

Protocol: Reverse Transfection of Adherent Cells with siRNA in 384-Well Plates

This protocol is provided as a starting point for optimization of siRNA transfection of adherent cells in a single well of a 384-well plate using the RNAi Human/Mouse Starter Kit. Starting points for optimizing transfection in other formats are listed in Table 3 on page 19. In this protocol, cell plating and transfection are performed on the same day.

Important points before starting

- Cells should be in optimal physiological condition at the time of transfection. The amount of cells seeded depends on the cell type and time of analysis.
- To prepare siRNAs provided in this kit: Add 250 µl RNase-free water (provided) to the lyophilized siRNA (5 nmol) to obtain a 20 µM solution.

  Perform your experiment or store at –20°C. Avoid repeated freeze-thaw cycles by aliquoting suitable amounts prior to storage.

Procedure

1. **Spot 3.125 ng siRNA in a volume of 1–3 µl into a single well of a 384-well plate (this will give a final siRNA concentration of 5 nM after addition of cells to complexes in step 4).**

   Note: After this step, siRNA may be stored at –20°C for long time periods. siRNA may be stored in solution or may be dried in the plate at room temperature (15–25°C) before storage.

2. **Add 0.5 µl of HiPerFect Transfection Reagent to 9.5 µl of culture medium without serum. Add the diluted HiPerFect Transfection Reagent to the prespotted siRNA.**

   Note: To ensure accurate pipetting, diluted HiPerFect Reagent should be prepared in a larger volume for use in multiple wells (see "Transfection in multiwell plates — preparing a master mix" on page 20).

   IMPORTANT: The amount of HiPerFect Transfection Reagent and siRNA required for optimal performance may vary, depending on the cell line and gene target. For details on optimization of the siRNA to HiPerFect Transfection Reagent ratio, see page 15.

3. **Incubate for 5–10 min at room temperature (15–25°C) to allow formation of transfection complexes.**

4. **Seed 4000–10,000 cells in 40 µl of an appropriate culture medium (containing serum and antibiotics) into the well, on top of the siRNA–HiPerFect Reagent transfection complexes.**

5. **Incubate the cells with the transfection complexes under their normal growth conditions and monitor gene silencing after an appropriate time (e.g., 6–72 h after transfection, depending on experimental setup). Change the medium as required.**

   Note: The optimal incubation time for gene silencing analysis depends on cell type, the gene targeted, and the method of analysis. This can be determined by performing a time-course experiment.

   Note: If monitoring transfection efficiency using AllStars Hs Cell Death Control siRNA, cells should be incubated for 72 hours prior to observation by light microscopy.

Protocol: Large-Scale Transfection of Adherent Cells with siRNA in 100 mm Dishes

This protocol is provided as a starting point for optimization of siRNA transfection of adherent cells in a 100 mm dish. Starting points for optimizing transfection in other formats are listed in Table 3 on page 19. In this protocol, cell plating and transfection are performed on the same day. For a few sensitive cell types, it may be necessary to plate the cells the day before transfection (as in the Traditional Protocol on page 24).

Important points before starting

- Cells should be in optimal physiological condition on the day of transfection. The optimal amount of cells seeded depends on the cell type and time of analysis.
- To prepare siRNAs provided in this kit: Add 250 µl RNase-free water (provided) to the lyophilized siRNA (5 nmol) to obtain a 20 µM solution.

  Perform your experiment or store at –20°C. Avoid repeated freeze-thaw cycles by aliquoting suitable amounts prior to storage.

Procedure

1. **Shortly before transfection, seed 2–4 x 10^6 cells per 100 mm dish in 7 ml of an appropriate culture medium containing serum and antibiotics.**
2. **For the short time until transfection, incubate the cells under normal growth conditions (typically 37°C and 5% CO_2).**

   Cells may alternatively be seeded after step 3 of this protocol.
3. **Dilute 600 ng siRNA in 1 ml culture medium without serum (this will give a final siRNA concentration of 5 nM after adding complexes to cells in step 5). Add 40 µl of HiPerFect Transfection Reagent to the diluted siRNA and mix by vortexing.**

   IMPORTANT: The amount of HiPerFect Transfection Reagent and siRNA required for optimal performance may vary, depending on the cell line and gene target. For details on optimization of the siRNA to HiPerFect Transfection Reagent ratio, see page 15.
4. **Incubate the samples for 5–10 min at room temperature (15–25°C) to allow the formation of transfection complexes.**
5. **Add the complexes drop-wise onto the cells. Gently swirl the dish to ensure uniform distribution of the transfection complexes.**

6. **Incubate the cells with the transfection complexes under their normal growth conditions and monitor gene silencing after an appropriate time (e.g., 6–72 h after transfection, depending on experimental setup). Change the medium as required.**

   Note: The optimal incubation time for gene silencing analysis depends on the cell type, the gene targeted, and the method of analysis. This can be determined by performing a time-course experiment.

   Note: If monitoring transfection efficiency using AllStars Hs Cell Death Control siRNA, cells should be incubated for 72 hours prior to observation by light microscopy.

Protocol: Long-Term, siRNA-Mediated Gene Silencing

This protocol is for long-term siRNA transfection of adherent cells in a single well of a 24-well plate. It is provided as a starting point for optimization of long-term siRNA transfection in mammalian cells.

Important points before starting

- Cells should be in optimal physiological condition on the day of transfection. The optimal confluency for transfection of adherent cells using this protocol is 50–80%. The amount of cells seeded depends on the cell type and time of analysis.
- To prepare siRNAs provided in this kit: Add 250 µl RNase-free water (provided) to the lyophilized siRNA (5 nmol) to obtain a 20 µM solution.

  Perform your experiment or store at –20°C. Avoid repeated freeze-thaw cycles by aliquoting suitable amounts prior to storage.
- This protocol requires sterile PBS and Trypsin/EDTA (not supplied).
  - Sterile 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)
  - Trypsin/EDTA (Hanks' Balanced Salt Solution containing 0.05% (w/v) porcine trypsin and 0.5 mM EDTA, or another appropriate trypsin or enzymatic solution)

Procedure

1. **The day before the first transfection, seed 2–8 x 10^4 cells per well of a 24-well plate in 0.5 ml of an appropriate culture medium containing serum and antibiotics.**
2. **Incubate the cells under normal growth conditions (typically 37°C and 5% CO_2).**
3. **On the day of transfection, dilute 37.5 ng siRNA in 100 µl culture medium without serum (this will give a final siRNA concentration of 5 nM after adding complexes to cells in step 5). Add 3 µl of HiPerFect Transfection Reagent to the diluted siRNA and mix by vortexing.**

   IMPORTANT: The amount of HiPerFect Transfection Reagent and siRNA required for optimal performance may vary, depending on the cell line and gene target. For details on optimization of the siRNA to HiPerFect Transfection Reagent ratio, see page 15.
4. **Incubate the samples for 5–10 min at room temperature (15–25°C) to allow the formation of transfection complexes.**

5. **Add the complexes drop-wise onto the cells. Gently swirl the plate to ensure uniform distribution of the transfection complexes.**
6. **Incubate the cells with the transfection complexes under their normal growth conditions. After 6–24 h, change the medium.**

   Note: Depending on the cell type, a medium change may not be necessary. Instead it may be possible to leave the complexes on the cells until they reach confluency.
7. **When the cells become confluent, they are split and retransfected as follows (steps 7–13). Remove the medium and wash the cells with 500 µl PBS.**
8. **Add 100 µl Trypsin/EDTA and incubate until the cells detach. Observe the cells closely to avoid extended incubation with trypsin.**
9. **Add 900 µl medium containing serum and antibiotics to stop the trypsination.**
10. **Prepare transfection complexes as described in steps 3 and 4.**
11. **Dilute an aliquot of the trypsinated cells in a final volume of 500 µl medium containing serum and antibiotics and transfer them to a fresh well of a 24-well plate.**

    Note: The dilution factor is cell-type–specific and usually reflects the doubling time of the cell type.
12. **Add the complexes drop-wise onto the cells. Gently swirl the plate to ensure uniform distribution of the transfection complexes.**
13. **Incubate the cells with the transfection complexes under their normal growth conditions. After 6–24 h, change the medium.**

    Note: Depending on the cell type, a medium change may not be necessary. Instead it may be possible to leave the complexes on the cells until they reach confluency or until analysis.
14. **When the cells become confluent and need to be split, repeat the procedure from step 7 onward.**
15. **Monitor gene silencing after an appropriate time.**

    Note: The optimal number of splitting cycles and transfections before gene silencing analysis depends on the gene targeted and the phenotype under study. This can be determined by performing a time-course experiment. Using this procedure, efficient knockdown without cytotoxicity has been observed up to 2 weeks after the initial transfection.

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 HiPerFect Transfection Reagent:siRNA ratio	Although fixed volumes of HiPerFect Transfection Reagent usually work very well with a range of siRNA concentrations, it could occur that the overall charge of the complexes is negative, neutral, or strongly positive, which can lead to inefficient adsorption to the cell surface. For optimal adsorption, complexes should be weakly positive. Optimize the HiPerFect Transfection Reagent to siRNA ratio using Table 1, page 16 or perform systematic titrations of HiPerFect Transfection Reagent.
b)	Suboptimal cell density	If cell density at the time of adding HiPerFect Transfection Reagent–siRNA complexes is not at an optimal level, cells may not be in the optimal growth phase for transfection. This can lead to insufficient uptake of complexes into the cells or inefficient processing of the siRNA. For adherent cells, the optimal confluency for transfection of siRNA is 50–80% if using the Traditional Protocol (page 24).
c)	Poor siRNA quality	siRNA should be of high quality, as impurities can lower transfection efficiency. HPP Grade siRNA from QIAGEN is >90% pure and provides efficient gene silencing.
Excessive cell death		
a)	Concentration of HiPerFect–siRNA complexes is too high	Decrease the amount of HiPerFect–siRNA complexes added to cells.

		Comments and suggestions
b)	Cells are stressed	Avoid stressing cells with temperature shifts and long periods without medium during washing steps. It is particularly important for transfection of siRNA 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 50–80% if using the Traditional Protocol (page 24).
c)	Poor siRNA quality	siRNA should be of high quality, as impurities can lower transfection efficiency. HPP Grade siRNA from QIAGEN is >90% pure and provides efficient gene silencing.
d)	Key gene is silenced	If the gene targeted is important for the survival of the cell, silencing this gene may lead to cell death.
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 the incubation time between seeding and complex addition consistent between experiments.
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 and morphology, and are less susceptible to 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)	Concentration of siRNA is too low	Increase siRNA concentration used in transfection.

		Comments and suggestions
No or very small gene silencing effect		
a)	Design of siRNA suboptimal	The design of an siRNA can have a large effect on its gene silencing efficiency. For predesigned siRNA for every human, mouse, and rat gene, visit www.qiagen.com/GeneGlobe.
b)	Incubation time after transfection is too short	The gene silencing effect observed at 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. QIAGEN offers siRNA that has been functionally tested for specific gene silencing (see ordering information, page 41).

Appendix: Protocol for Western Blot Analysis for Detection of MAPK1

This protocol is for detection of MAPK1 protein by western blotting using the Tag·100 Antibody (cat. no. 34680). It is optimized for a Mini Protean Chamber (Bio-Rad) and is based on standard western blotting techniques (28). A detailed protocol for western blot analysis can be found in the *QIA*express® *Detection and Assay Handbook* supplied with the Tag·100 Antibody and is available at www.qiagen.com/HB/QIAexpressDetectionAndAssay. Further information about protein analysis can be found at www.qiagen.com/literature/BenchGuide.

Procedure

1. **Pour a 12% denaturing polyacrylamide* gel.**
2. **Prepare lysates from the cells under study using standard procedures and denaturing conditions (28). Heat the cell lysates in an appropriate SDS-sample buffer for 5 min at 95°C, then place on ice.**

   For detailed information about separation of proteins by SDS-PAGE, refer to the *QIA*express *Detection and Assay Handbook*.
3. **Spin down any condensed liquid and load approximately 3 µg total cell protein in each well.**
4. **Run the gel for 15 min at 80 V, then increase to 100 V for approximately 60–75 min.**
5. **Blot the gel on an appropriate membrane (e.g., Protran® Nitrocellulose, Schleicher and Schuell).**

   For detailed information about western transfer, refer to the *QIA*express *Detection and Assay Handbook*.
6. **Block the membrane by gently shaking in 5% (w/v) fat-free milk made in TBS* (Tris-buffered saline)-Tween® for 60 min at room temperature (15–25°C).**

   TBS-Tween is 0.02% (v/v) Tween 20 in 10 mM Tris·Cl, 150 mM NaCl, pH 7.5.
7. **Wash the blot as follows:**

   1 x 5 min in TBS

   2 x 5 min in TBS-Tween

   1 x 5 min in TBS

* 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.

8. **Dilute the Tag·100 Antibody 1/1000 in 5% (w/v) fat-free milk made in TBS-Tween and gently shake the membrane in the diluted antibody solution for 60 min at room temperature or overnight at 4°C.**

   Note: A 1/2000 dilution of monoclonal anti-a-tubulin antibody (Clone B-5-1-2, product no. T5168, Sigma) can be used for staining in parallel as a positive control.

9. **Wash the blot as described in step 7.**

10. **Perform detection using standard procedures (e.g., ECL Western Blot detection reagents, GE Healthcare).**

    For detailed information about detection, go to www.qiagen.com/literature/BenchGuide.

    Note: The expected size of MAPK1 is approximately 41 kDa.

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.

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Ordering Information

Product	Contents	Cat. no.
RNAi Human/Mouse Starter Kit	HiPerFect Transfection Reagent, AllStars Negative Control siRNA, Hs/Mm_MAPK1 Control siRNA, AllStars Hs Cell Death Control siRNA, RNase-Free Water	301799
Accessories		
Rn_Mapk1 Control siRNA (5 nmol)	Positive control siRNA for use in rat cells; provides proven high knockdown of the rat MAPK1 gene	1027277
AllStars Mm/Rn Cell Death Control siRNA (1 nmol)	1 nmol positive cell death phenotype control for mouse and rat cells	SI049 39025
HiPerFect Transfection Reagent (0.5 ml)	HiPerFect Transfection Reagent for up to 166 transfections in 24-well plates	301704
HiPerFect Transfection Reagent (1 ml)	HiPerFect Transfection Reagent for up to 333 transfections in 24-well plates	301705
HiPerFect Transfection Reagent (4 x 1 ml)	HiPerFect Transfection Reagent for up to 1332 transfections in 24-well plates	301707
HiPerFect Transfection Reagent (100 ml)	HiPerFect Transfection Reagent for transfections in up to 1388 96-well plates	301709
FlexiTube siRNA	1 nmol siRNA delivered in tubes (HP Validated siRNA or HP GenomeWide siRNA); minimum order of 4 siRNAs	Varies*
FlexiTube GeneSolution	4 siRNAs recommended for your gene at GeneGlobe; siRNAs delivered in tubes in 1-nmol amounts	Varies*

* Visit www.qiagen.com/GeneGlobe to search for and order these products.

Ordering Information

Product	Contents	Cat. no.
FlexiPlate siRNA	Custom siRNA set for customer-specified genes and siRNA controls; minimum order 36 siRNAs; 0.1 nmol, 0.25 nmol, or 1 nmol scale; plate layout chosen by the customer at GeneGlobe	Varies*
HP GenomeWide siRNA	Predesigned siRNA for each gene of the human, mouse, and rat genomes, available in 5 nmol or 20 nmol amounts and with a range of fluorescent labels and modification options	Varies*
HP Validated siRNA	Predesigned siRNA that has been functionally tested by quantitative, real-time RT-PCR, available in 5 nmol or 20 nmol amounts and with a range of fluorescent labels and modification options	Varies*
Tag·100 Antibody, BSA-free (100 µg)	100 µg mouse anti-Tag·100 antibody, recognizes endogenous mammalian MAPK1	34680
QuantiTect Primer Assays	For 200 x 50 µl reactions: lyophilized primer mix	Varies*
Hs_MAPK1_1_SG QuantiTect Primer Assay	Lyophilized mix of forward and reverse primers specific for the human MAPK1 gene	QT000 65933
Mm_Mapk1_1_SG QuantiTect Primer Assay	Lyophilized mix of forward and reverse primers specific for the mouse MAPK1 gene	QT001 33840
Rn_Mapk1_1_SG QuantiTect Primer Assay	Lyophilized mix of forward and reverse primers specific for the rat MAPK1 gene	QT001 90379

* Visit www.qiagen.com/GeneGlobe to search for and order these products.

Ordering Information

Product	Contents	Cat. no.
QuantiTect SYBR Green RT-PCR Kit (200)*	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect SYBR Green RT-PCR Master Mix (providing a final concentration of 2.5 mM $MgCl_2$), 100 µl QuantiTect RT Mix, 2 x 2 ml RNase-Free water	204243
QuantiTect SYBR Green PCR Kit (200)*	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect SYBR Green PCR Master Mix (providing a final concentration of 2.5 mM $MgCl_2$), 2 x 2 ml RNase-Free water	204143
QuantiFast SYBR Green PCR Kit (80)*	For 80 x 25 µl reactions: 1 ml 2x QuantiFast SYBR Green PCR Master Mix (contains ROX dye), 2 ml RNase-Free Water	204052
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
QuantiTect Reverse Transcription Kit (50)*	For 50 x 20 µl reactions: gDNA Wipeout Buffer, Quantiscript® Reverse Transcriptase, Quantiscript RT Buffer, RT Primer Mix, and RNase-Free Water	205311
RNeasy Mini Kit (50)†	50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74104
FastLane Cell cDNA Kit (50)	Buffer FCW, Buffer FCP, and components for 50 x 20 µl reverse-transcription reactions	215011
FastLane Cell SYBR Green Kit (200)	FastLane Cell One-Step Buffer Set, 2x QuantiTect SYBR Green RT-PCR Master Mix, and QuantiTect RT Mix	216213

* Larger kit sizes available; please inquire.

† RNeasy Kits are also available in micro, midi, maxi, and 96-well formats; please inquire.

The RNAi Human/Mouse Starter Kit, siRNA products, HiPerFect Transfection Reagent, RNeasy Mini Kit and Tag·100 Antibody are intended for molecular biology applications. They are neither intended for the diagnosis, prevention, or treatment of disease, nor have they been validated for such use either alone or in combination with other products. QuantiTect products, QuantiFast products, and FastLane products are intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Notes

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