September 2013

Guidelines for miRNA mimic and miRNA inhibitor experiments

For miRNA research

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Introduction

microRNAs (miRNAs) are a class of endogenous small RNA molecules with similar characteristics to siRNAs. In recent years, it has been discovered that miRNAs play a role in many diverse biological processes, such as development, differentiation, and apoptosis. Misregulation of miRNA expression is reported to be associated with several cancers and other diseases.

The miRNA system is an endogenous mechanism of regulation of gene expression. Mature miRNAs contribute to the regulation of endogenous genes, primarily by translational repression. In addition, miRNAs can mediate mRNA destruction by rapid deadenylation and/or decapping. Naturally occurring miRNA-binding sites are typically found in the 3' untranslated regions (UTRs) of target mRNAs. Partial complementarity between the target binding site and the miRNA has made positive identification of true binding sites difficult and imprecise.

Principle and procedure

Transfection of miRNA mimics or inhibitors is a technique used to identify the targets and roles of particular miRNAs. miRNA mimics are chemically synthesized miRNAs which mimic naturally occurring miRNAs after transfection into the cell. miRNA inhibitors are single-stranded, modified RNAs which, after transfection, specifically inhibit miRNA function. Reduced gene expression after transfection of an miRNA mimic or increased expression after transfection of an miRNA inhibitor provides evidence that the miRNA under study is involved in regulation of that gene. Alternatively, the role of miRNAs in various pathways can be studied by examination of a specific phenotype following miRNA mimic or inhibitor transfection.

This handbook provides protocols for miRNA mimic and inhibitor transfection. These protocols are intended as starting points for miRNA mimic and inhibitor experiments as, depending on the miRNA and analysis method used, experimental conditions may require further optimization. The handbook also describes setup and optimization of such experiments, and recommends appropriate controls.

Description of protocols

This handbook contains 5 protocols. Of these, 3 are for transfection of adherent cells, and 2 are specifically for transfection of HeLa cells. The HeLa cell protocols may be used for other adherent cell types. However, some optimization may be required. In all the protocols, cell seeding and transfection are carried out on the same day. This is quicker and saves labor in comparison to traditional protocols in which cells are seeded the day before transfection.

Protocols for miRNA mimic or inhibitor transfection

A protocol for miRNA mimic or inhibitor transfection in 24-well plates is provided on page 18. In this protocol, cells are seeded in wells first, followed by addition of mimic/inhibitor–reagent complexes. When using 24-well plates, we recommend that transfection is performed in this order to ensure optimal mixing of cells and complexes. However, reverse transfection, where complexes are added to wells first and then cells are added on top of complexes, can be performed if desired. To perform a reverse transfection, simply change the order in which cells and complexes are added to the plate.

A protocol for miRNA mimic or inhibitor reverse transfection in 96-well plates is provided on page 20. In this protocol, miRNA mimic or inhibitor is added to wells followed by the addition of HiPerFect Transfection Reagent. After complex formation, cells are added to the wells. Reverse transfection is rapid and convenient, and is frequently used for high-throughput formats.

A protocol for cotransfection of miRNA mimic and miRNA inhibitor in 24-well plates is provided on page 22. This is a reverse transfection, with complexes added to wells first and cells added on top of complexes.

Protocols for plasmid DNA and miRNA mimic/inhibitor cotransfection

Many miRNA experiments involve cotransfection of an miRNA mimic and/or inhibitor together with a plasmid DNA vector in which miRNA-binding sites are fused to a reporter gene, such as luciferase. Two protocols are provided for these types of experiments in HeLa cells. These protocols are also suitable for use with other adherent cells, but may require further optimization. A protocol is provided for cotransfection of plasmid DNA with an miRNA mimic or an miRNA inhibitor (page 24). In addition, a protocol is provided for cotransfection of plasmid DNA with both an miRNA mimic and an miRNA inhibitor simultaneously (page 26).

The TransFect Protocol Database

Cell-type–specific protocols for transfection of small RNA, such as siRNA and miRNA mimics/inhibitors, are available at the Transfect Protocol Database (www.qiagen.com/TransFect). Simply enter the cell type and nucleic acid of interest to receive a ready-to-print protocol.

Optimization of miRNA experiments

The amount of miRNA mimic/inhibitor needed to efficiently downregulate a target gene or inhibit miRNA function can vary greatly, depending on the miRNA, the cell line, and the chosen analysis method. To determine the concentration that provides optimal results, optimization experiments using varying mimic/inhibitor concentrations should be performed.

miRNA mimics can inhibit target protein expression at a final concentration as low as 0.5 nM. However, a higher concentration may be required, especially if performing downstream analysis at the protein level. miRNA inhibitors have been shown to inhibit miRNA function at a concentration of 50 nM. Lower inhibitor concentrations may also be effective.

In addition to optimization of concentration, time-course experiments may also be necessary to determine the optimal time after transfection for analysis of results. miRNA mimic or inhibitor effects often do not lead to an immediate change in transcript or protein levels.

Downstream analysis

Downstream analysis of the effect of miRNA mimic/inhibitor transfection is often performed using one of the following strategies:

- A plasmid vector which carries a reporter gene such as luciferase and one or more miRNA binding sites in the 3' UTR is used as an miRNA target. miRNA mimic and/or inhibitor is cotransfected with the vector. After transfection, a reporter assay, such as a luciferase assay, is performed. The effect of the mimic/inhibitor is determined by comparing this to the result from cells transfected with the vector alone.
- The expression of an endogenous gene, which is known to be a target of the miRNA under study, is measured after mimic/inhibitor transfection. The effect of the mimic/inhibitor is determined by comparing this result with the gene expression in untransfected cells or cells transfected with a negative control. Gene expression is often measured at the protein level, for example, by western blot, as miRNAs often inhibit translation of their target genes and do not cause degradation of the target transcript. This means that the effect of an miRNA mimic or inhibitor can often not be determined using quantitative, real-time PCR.

Determining miRNA mimic or inhibitor effects at the protein level

If using an endogenous gene target for downstream analysis, the effects of miRNA mimics/inhibitors should be determined at the protein level. Testing the chosen detection method together with a positive control allows identification of optimal experimental conditions. A time-course experiment will identify the optimal time to analyze the effect, as this time point will differ between miRNAs and also depends on the turnover of the target protein.

miRNA inhibition of a target protein can differ greatly, depending on the model system used. The same target protein can be regulated differently by the same miRNA in different cell types. The effects can also depend on the developmental or cell cycle stage, and on growth conditions. Expression of other miRNAs may also be required to achieve synergistic effects necessary to efficiently regulate a target.

The use of miRNA inhibitors to investigate the effect of endogenously expressed miRNAs can be especially challenging, as many target genes are regulated by more than one miRNA. It may be necessary to inhibit more than one miRNA to see an effect on the target protein. If possible, it is preferable to choose a cell type in which only one or few miRNAs which can potentially regulate the chosen target protein are expressed.

It is also important to note that, while predictions of miRNAs targets are becoming increasingly accurate, it is nevertheless possible that a particular target is not regulated by the miRNA of interest or is not regulated by the miRNA under the chosen experimental conditions. The target may also be regulated by multiple miRNAs and an effect may not be observed when using a single mimic or inhibitor.

Controls

The use of appropriate controls is essential for the correct interpretation of results from experiments using miRNA mimics or inhibitors. Every experiment should include a suitable positive and negative control. Additional controls may also be necessary to enable interpretation of results or troubleshooting. The following paragraphs provide descriptions of control experiments.

miRNA mimic experiments — positive control

Transfection of a positive control mimic can be used to confirm that the experimental system is working as expected (i.e., that the mimic is efficiently transfected and causes downregulation of the target). This control can also be used in optimization experiments where varying concentrations are used for transfection to determine the concentration that provides optimal results. A positive control should be routinely transfected in every experiment using miRNA mimics to confirm that conditions remain optimal.

Syn-hsa-miR-1 miScript miRNA Mimic (cat. no. MSY0000416) is a synthetic miRNA mimic of the human miRNA hsa-miR-1. hsa-miR-1 is only expressed in muscle cells and is not expressed in most other cell types. If hsa-miR-1 is not expressed in the cell type under study, then transfection of this mimic and subsequent analysis of its target can be performed as a positive control experiment. To check whether hsa-miR-1 is expressed in the chosen cell type, we recommend performing a real-time PCR experiment using the miScript PCR System and the miScript Primer Assay for hsa-miR-1 (Hs_miR-1_2 miScript Primer Assay (100), cat. no. MS00008358).

Transfection of Syn-hsa-miR-1 miScript miRNA Mimic should result in downregulation of a target of this mimic if the system is working optimally. The effect of transfection of Syn-hsa-miR-1 miScript miRNA Mimic can be analyzed by quantifying expression of an endogenous target of hsa-miR-1, such as HDAC4. Alternatively, a reporter vector which carries a hsa-miR-1 binding site

can be used as a target. In this case, the vector should be cotransfected with Syn-hsa-miR-1 miScript miRNA Mimic. Measurement of reporter activity should show a decrease in activity after cotransfection compared to the negative control (see below).

miRNA mimic experiments — negative control

A negative control should be transfected in every experiment and will indicate if results are nonspecific. Comparison of results from the negative control with results from the miRNA mimic under study can be used to confirm that the observed results are specific to the miRNA mimic under study. Results from the negative control should also be compared to results from untransfected cells. Gene expression and phenotype should be similar in both untransfected cells and cells transfected with the negative control.

QIAGEN recommends AllStars Negative Control siRNA (cat. no. 1027280) for use as a negative control. AllStars Negative Control siRNA is the most thoroughly tested and validated negative control siRNA currently available (for comprehensive data, visit www.qiagen.com/AllStars). It has no homology to any known mammalian gene. Since miRNA mimics and siRNAs are chemically very similar and usually differ only in sequence, a negative control siRNA can also be used as a negative control miRNA mimic.

In experiments in which a reporter vector is used for downstream analysis, the negative control should be a cotransfection of AllStars Negative Control siRNA and the reporter vector.

miRNA inhibitor experiments — positive control

In experiments involving transfection of miRNA inhibitors, detection of the inhibitor effect is often complicated by the presence of other miRNAs in the cell which interact with the same target gene. For this reason, we recommend a positive control experiment in which cells are cotransfected with both Anti-hsa-miR-1 miScript miRNA Inhibitor (a hsa-miR-1 inhibitor, cat. no. MIN0000416) and Syn-hsa-miR-1 miScript miRNA Mimic (a hsa-miR-1 mimic, cat. no. MSY0000416). In parallel, Syn-hsa-miR-1 miScript miRNA Mimic should also be transfected alone (see "miRNA mimic experiments — positive control"). If the Syn-hsa-miR-1 miScript miRNA Mimic alone does not show any effect, then the miRNA inhibitor positive control cannot be interpreted.

Cotransfection of mimic and inhibitor should result in an increase in expression when compared to the mimic alone. This confirms that the inhibitor is effectively inhibiting the mimic, resulting in upregulation of the hsa-miR-1 gene target.

In experiments in which a reporter vector will be used for downstream analysis, the vector, mimic, and inhibitor should all be cotransfected together and, in parallel, the vector and mimic should be cotransfected (see "miRNA mimic experiments — positive control"). Transfection of vector, mimic, and inhibitor

should result in an increase in expression when compared to transfection of vector and mimic. This confirms that the inhibitor is effectively inhibiting the mimic, resulting in upregulation of the reporter.

Two protocols are provided for this positive control experiment, depending on the hsa-miR-1 target used. For detection of an endogenous target, such as HDAC4, a high concentration of mimic is recommended, and a protocol for cotransfection of mimic and inhibitor is provided (page 22). For vector-based reporter assays, a lower concentration of mimic is recommended, and a protocol for cotransfection of vector, mimic, and inhibitor is provided (page 26).

miRNA inhibitor experiments — negative control

A negative control should be transfected in every inhibitor experiment and will indicate if results are nonspecific. Results achieved after transfection of this control should be similar to results from untransfected cells. Comparison of results from the negative control with results from the inhibitor under study can be used to confirm that the observed results are specific to the inhibitor under study.

QIAGEN recommends miScript Inhibitor Negative Control, which targets the sequence of AllStars Negative Control siRNA, and has no homology to any known mammalian gene.

In experiments in which an endogenous gene will be used for downstream analysis, the negative control should be a transfection of miScript Inhibitor Negative Control. In experiments in which a reporter vector will be used for downstream analysis, the negative control should be a cotransfection of miScript Inhibitor Negative Control and the reporter vector.

Transfection control

For optimal results, transfection efficiency should be as high as possible. AllStars Hs Cell Death Control siRNA is an siRNA blend which targets essential human genes. Transfection of this control causes a high degree of cell death which can be observed by light microscopy.

When performing start-up experiments or working with 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 using AllStars Hs Cell Death Control siRNA (cat. no. 1027298). Transfection conditions that result in the greatest degree of cell death in comparison to transfection with a negative control can be maintained in future experiments. This control should be performed for optimization and start-up experiments and can be used as a routine transfection control in every experiment.

AllStars Hs Cell Death Control siRNA is suitable for use in both miRNA mimic and miRNA inhibitor experiments. This is because transfection properties of small RNA molecules such as siRNAs, miRNA mimics, or miRNA inhibitors are very similar.

For routine transfection controls in experiments in which a reporter vector will be used for downstream analysis, we recommend cotransfection of AllStars Hs Cell Death Control siRNA and the reporter vector.

Mock-transfection control

Mock-transfected cells go through the transfection process without addition of miRNA mimic/inhibitor (i.e., cells are treated with transfection reagent only). This control should be performed during start-up experiments to determine whether the transfection reagent or process causes nonspecific or cytotoxic effects.

Untransfected cells control

Gene expression or phenotypic analysis should be carried out on cells that have not been treated to allow measurement of the normal phenotype or basal level of gene expression. Results from untreated cells can be used for comparison with results from all other samples. Untreated cells should be analyzed in every experiment.

Unregulated-vector control

If a reporter vector is used in which the reporter gene is regulated by an miRNA binding site in its 3' UTR, we recommend always transfecting an unregulated reporter as an additional control in each experiment (i.e., transfecting the reporter vector without miRNA binding site in its 3' UTR). Comparison of experimental results with results from this control will indicate whether effects are caused by miRNA regulation, or whether they are nonspecific and due to some other vector-related factor.

Normalization controls

To eliminate experimental variations that could otherwise result in inaccurate quantification, an unregulated gene should be used for normalization. In experiments in which a reporter vector will be used for downstream analysis, this could be a reporter on a separate vector that is cotransfected in every experiment. Alternatively, a second reporter that is expressed from the same vector as the miRNA target, but that is not itself regulated by the miRNA, can be used for this purpose. If the effect of the miRNA mimic or inhibitor will be determined by analyzing the expression of endogenous targets, we recommend using an unregulated endogenous gene such as tubulin or β-actin for normalization.

Table 1. Recommended controls for miRNA experiments using an endogenous gene for downstream analysis

Experiment	Positive control	Negative control
miRNA mimic experiments	■ hsa-miR-1 mimic	■ AllStars Negative Control siRNA
miRNA inhibitor experiments	■ hsa-miR-1 mimic and hsa-miR-1 inhibitor* ■ hsa-miR-1 mimic	■ miScript Inhibitor Negative Control
All miRNA experiments	■ AllStars Hs Cell Death Control siRNA ■ Untransfected cells control ■ Mock-transfection control ■ Normalization control	

* These controls are cotransfections.

Table 2. Recommended controls for miRNA experiments using a reporter vector for downstream analysis

Experiment	Positive control	Negative control
miRNA mimic experiments	■ hsa-miR-1 mimic and vector*	■ AllStars Negative Control siRNA and vector*
miRNA inhibitor experiments	■ hsa-miR-1 mimic and hsa-miR-1 inhibitor and vector* ■ hsa-miR-1 mimic and vector*	■ miScript Inhibitor Negative Control and vector*
All miRNA experiments	■ AllStars Hs Cell Death Control siRNA and vector* ■ Untransfected cells control ■ Mock-transfection control ■ Normalization control ■ Unregulated-vector control	

* These controls are cotransfections.

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 medium
- miRNA mimic/inhibitor of interest. miScript miRNA Mimics and Inhibitors can easily be ordered at www.qiagen.com/GeneGlobe/miRNAproducts. miScript miRNA Mimics and Inhibitors from QIAGEN are provided lyophilized and must be resuspended as follows:

  Briefly centrifuge tubes prior to opening as some of the product may have been dislodged during shipping. Resuspend with an appropriate volume of RNase-free water (provided). To achieve a final concentration of 20 µM (recommended), add 250 µl water to 5 nmol miScript miRNA Mimic, miScript miRNA Inhibitor, or AllStars Hs Cell Death Control siRNA. Vortex the tube until the lyophilized miRNA has dissolved. Store at –20°C. Repeated freeze–thaw cycles will not interfere with the performance of miScript miRNA Mimic, miScript miRNA Inhibitor, or AllStars Hs Cell Death Control siRNA as long as RNase-free conditions are strictly maintained.
- Transfection reagent. We recommend HiPerFect Transfection Reagent for miRNA mimic/inhibitor transfection and Attractene Transfection Reagent for plasmid DNA–miRNA mimic/inhibitor cotransfection (see page 36 for ordering information).
- A detection system to analyze the effect of transfection of miRNA mimic/inhibitor (e.g., a vector carrying a reporter gene and an miRNA binding site, or antibodies against an miRNA target for use in western blotting).

Important Notes

Optimizing miRNA transfection

We recommend HiPerFect Transfection Reagent for transfection of miRNA mimic and/or inhibitor, and Attractene Transfection Reagent for cotransfection of miRNA mimic/inhibitor and plasmid DNA. To achieve the best results in miRNA mimic/inhibitor transfection of adherent cells, we recommend optimizing the following parameters. For more details on optimizing transfection, refer to the *HiPerFect Transfection Reagent Handbook* (www.qiagen.com/HB/HiPerFect) and to the *Attractene Transfection Reagent Handbook* (www.qiagen.com/HB/AttracteneTransfectionReagent_EN).

Amount of miRNA mimic/inhibitor

The amount of miRNA used is critical for efficient transfection and gene silencing. The recommended starting concentration for transfection in 24-well plates is 5 nM for miRNA mimic and 50 nM for miRNA inhibitor. A pipetting scheme for optimizing transfection of adherent cells in 24-well plates is shown in Table 3 (page 15).

Ratio of HiPerFect Transfection Reagent to miRNA mimic/inhibitor

The ratio of HiPerFect Transfection Reagent to miRNA mimic/inhibitor should be optimized for every new cell type and miRNA combination used. As a starting point for optimization when using 24-well plates, we recommend 5 nM miRNA mimic/50 nM miRNA inhibitor and 3 µl HiPerFect Transfection Reagent for adherent cells. To optimize miRNA transfection in 24-well plates, prepare separate transfection mixtures according to Table 3 (page 15).

Please note that Table 3 is intended only as a guideline for starting amounts of miRNA 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 3. Pipetting scheme for optimizing miRNA mimic/inhibitor transfection in 24-well plates*

Volume (conc.) of 20 µM stock miRNA mimic	0.3 µl (10 nM)	0.3 µl (10 nM)	0.3 µl (10 nM)
Volume (conc.) of 20 µM stock miRNA inhibitor	3 µl (100 nM)	3 µl (100 nM)	3 µl (100 nM)
Volume of HiPerFect Reagent	1.5 µl	3 µl	4.5 µl
Volume (conc.) of 20 µM stock miRNA mimic	0.15 µl (5 nM)	**0.15 µl (5 nM)**	0.15 µl (5 nM)
Volume (conc.) of 20 µM stock miRNA inhibitor	1.5 µl (50 nM)	**1.5 µl (50 nM)**	1.5 µl (50 nM)
Volume of HiPerFect Reagent	1.5 µl	**3 µl**	4.5 µl
Volume (conc.) of 20 µM stock miRNA mimic	0.03 µl (1 nM)	0.03 µl (1 nM)	0.03 µl (1 nM)
Volume (conc.) of 20 µM stock miRNA inhibitor	0.3 µl (10 nM)	0.3 µl (10 nM)	0.3 µl (10 nM)
Volume of HiPerFect Reagent	1.5 µl	3 µl	4.5 µl

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

Optimizing DNA–miRNA transfection

When optimizing transfection of miRNA mimic/inhibitor and a reporter vector, the amount of DNA and Attractene Transfection Reagent should be optimized first. Table 4 shows a pipetting scheme for optimizing transfection of cells in 24-well plates. This scheme shows 9 conditions which we recommend to test when optimizing transfection. As a starting point, we recommend using 0.3 µg DNA and 1.5 µl Attractene Reagent (in bold in Table 4). For transfection using other culture formats, refer to Table 5.

Table 4. Pipetting scheme for optimizing DNA transfection in 24-well plates*

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

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

Table 5. Starting points for transfection in different formats

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

Amount of miRNA mimic/inhibitor

Once the ratio of DNA reporter and Attractene Transfection Reagent is optimized, the correct amount of miRNA mimic/inhibitor should be determined. The recommended starting concentration for transfection in 24-well plates is 5 nM for miRNA mimic and 50 nM for miRNA inhibitor.

Cell density at transfection

The optimal cell confluency for transfection should be determined for every new cell type to be transfected and kept constant in future experiments. This is achieved by counting cells before seeding. 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 is

shown in Table 6. For further information about optimizing transfection of small RNAs in various formats, refer to the *HiPerFect Transfection Reagent Handbook* (www.qiagen.com/HB/HiPerFect) or to the *Attractene Transfection Reagent Handbook* (www.qiagen.com/HB/AttracteneTransfectionReagent_EN).

Table 6. Recommended number of adherent cells to seed for different formats

Culture format	Cell number
384-well plate	4000–10,000
96-well plate	1–5 x 10^4
48-well plate	2–8 x 10^4
24-well plate	0.4–1.6 x 10^5
12-well plate	0.8–3 x 10^5
6-well plate	1.5–6 x 10^5
60 mm dish	0.3–1.2 x 10^6
100 mm dish	2–4 x 10^6

Transfection in multiwell plates — preparing a master mix

If you are performing transfection in multiwell plates, prepare a master mix of transfection complexes 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.

Protocol: Transfection of Adherent Cells with miRNA Mimics or miRNA Inhibitors in 24-Well Plates

This protocol is provided as a starting point for optimization of miRNA mimic or miRNA inhibitor transfection in a 24-well format. The amounts given are for one well of a 24-well plate. In this protocol, cell seeding and transfection are performed on the same day.

When using 24-well plates, we recommend that transfection is performed in the order described in this protocol, with cells seeded in wells first followed by addition of mimic/inhibitor–reagent complexes. This ensures optimal mixing of cells and complexes. However, reverse transfection, where complexes are added to wells first and then cells added on top of complexes, can be performed if desired. To perform a reverse transfection, simply change the order in which cells and complexes are added to the plate.

Important point 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.

Things to do before starting

- If you have ordered miRNA mimics or inhibitors from QIAGEN, they are delivered lyophilized and must be resuspended prior to transfection. To resuspend, follow the instructions provided on page 13.

Procedure

1. **Shortly before transfection, seed 0.4–1.6 x 10^5 cells per well of a 24-well plate in 500 µl 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 prepared after step 3 of this protocol.
3. **Dilute 0.15 µl miRNA mimic (20 µM stock) or 1.5 µl miRNA inhibitor (20 µM stock) in 100 µl culture medium without serum (this will give a final miRNA mimic concentration of 5 nM or a final miRNA inhibitor concentration of 50 nM after adding complexes to cells in step 5). Add 3 µl HiPerFect Transfection Reagent to the diluted miRNA mimic/inhibitor and mix by vortexing.**

   IMPORTANT: The amount of transfection reagent and miRNA mimic/inhibitor required for optimal performance may vary, depending on the cell line and gene target.

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 expression 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 analysis depends on the cell type, the gene targeted, and the method of analysis. This can be determined by performing a time-course experiment.

Protocol: Reverse Transfection of Adherent Cells with miRNA Mimics or miRNA Inhibitors in 96-Well Plates

This protocol is provided as a starting point for optimization of miRNA mimic or miRNA inhibitor transfection in a 96-well format. The amounts given are for one well of a 96-well plate. In this protocol, cell seeding and transfection are performed on the same day.

Important point 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.

Things to do before starting

- If you have ordered miRNA mimics or inhibitors from QIAGEN, they are delivered lyophilized and must be resuspended prior to transfection. To resuspend, follow the instructions provided on page 13.

Procedure

1. **Spot 0.05 µl miRNA mimic (20 µM stock) or 0.5 µl miRNA inhibitor (20 µM stock) in 1–3 µl of RNase-free water into a single well of a 96-well plate (this will give a final miRNA mimic concentration of 5 nM or a final miRNA inhibitor concentration of 50 nM after addition of cells to complexes in step 4).**

   Note: If preferred, miRNA mimic/inhibitor can be spotted in 25 µl of RNase-free water 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 miRNA mimic/inhibitor.**

   Note: To ensure accurate pipetting, diluted HiPerFect Reagent should be prepared in a larger volume for use in multiple wells. Then add 25 µl of the dilution to a single well.

   IMPORTANT: The amount of HiPerFect Transfection Reagent and miRNA mimic/inhibitor required for optimal performance may vary, depending on the cell line and gene target.

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 miRNA mimic/inhibitor–HiPerFect Reagent transfection complexes.**

5. **Incubate the cells with the transfection complexes under their normal growth conditions and monitor gene expression 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 analysis depends on the cell type, the gene targeted, and the method of analysis. This can be determined by performing a time-course experiment.

Protocol: Cotransfection of Adherent Cells with miRNA Mimics and miRNA Inhibitors in 24-Well Plates

This protocol is provided as a starting point for optimization of miRNA mimic and miRNA inhibitor cotransfection in a 24-well format. The amounts given are for one well of a 24-well plate. In this protocol, cell seeding and transfection are performed on the same day.

Important point 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.

Things to do before starting

- If you have ordered miRNA mimics or inhibitors from QIAGEN, they are delivered lyophilized and must be resuspended prior to transfection. To resuspend, follow the instructions provided on page 13.

Procedure

1. **Shortly before transfection, seed 0.4–1.6 x 10^5 cells per well of a 24-well plate in 500 µl 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 prepared after step 4 of this protocol.
3. **Dilute 0.15 µl miRNA mimic (20 µM stock) in 50 µl culture medium without serum (this will give a final miRNA mimic concentration of 5 nM after adding complexes to cells in step 5). Add 1.5 µl of HiPerFect Transfection Reagent to the diluted miRNA mimic and mix by vortexing.**

   IMPORTANT: The amount of transfection reagent and miRNA mimic required for optimal performance may vary, depending on the cell line and gene target.
4. **Dilute 1.5 µl miRNA inhibitor (20 µM stock) in 50 µl culture medium without serum (this will give a final miRNA inhibitor concentration of 50 nM after adding complexes to cells in step 5). Add 1.5 µl HiPerFect Transfection Reagent to the diluted miRNA inhibitor and mix by vortexing.**

IMPORTANT: The amount of transfection reagent and miRNA inhibitor required for optimal performance may vary, depending on the cell line and gene target.

5. **Incubate the samples for 5–10 min at room temperature (15–25°C) to allow the formation of transfection complexes.**
6. **Mix miRNA mimic–HiPerFect Reagent complexes from step 3 and miRNA inhibitor–HiPerFect Reagent complexes from step 4.**
7. **Add the complex mixture drop-wise onto the cells. Gently swirl the plate to ensure uniform distribution of the transfection complexes.**
8. **Incubate the cells with the transfection complexes under their normal growth conditions and monitor gene expression 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 analysis depends on the cell type, the gene targeted, and the method of analysis. This can be determined by performing a time-course experiment.

Protocol: Cotransfection of HeLa S3 Cells with Plasmid DNA and miRNA Mimic or Inhibitor in 24-Well Plates

This protocol is provided as a starting point for optimization of miRNA mimic or inhibitor and plasmid DNA cotransfection in HeLa S3 cells using Attractene Transfection Reagent. The amounts given are for one well of a 24-well plate. In this protocol, cell seeding and transfection are performed on the same day. For use with other adherent cell types, optimization of the protocol parameters will be necessary.

Important point 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.

Things to do before starting

- If you have ordered miRNA mimics or inhibitors from QIAGEN, they are delivered lyophilized and must be resuspended prior to transfection. To resuspend, follow the instructions provided on page 13.

Procedure

1. **Shortly before transfection, dilute 6 x 10^4 cells in 500 µl 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 prepared after step 4 of this protocol.
3. **Dilute 400 ng plasmid DNA and 0.14 µl miRNA mimic (20 µM stock) or 1.4 µl miRNA inhibitor (20 µM stock) in 60 µl medium without serum or antibiotics (this will give a final miRNA mimic concentration of 5 nM or a final miRNA inhibitor concentration of 50 nM after adding cells to complexes in step 7).**
   IMPORTANT: The amount of Attractene Transfection Reagent, plasmid DNA, and miRNA mimic/inhibitor required for optimal performance may vary, depending on the detection method.
4. **Add 1.5 µl Attractene Transfection Reagent. Mix by pipetting up and down.**
5. **Incubate for 10–15 min at room temperature (15–25°C) to allow the formation of transfection complexes. Then add the complexes to a single well of a 24-well plate.**

6. **Add the 500 µl cell culture from step 1 to the well. Gently swirl the plate to ensure uniform distribution of the transfection complexes.**
7. **Incubate the cells with the transfection complexes under their normal growth conditions and monitor gene expression after an appropriate time (e.g., 24–72 h after transfection, depending on experimental setup). Change the medium as required.**

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

Protocol: Cotransfection of HeLa S3 Cells with Plasmid DNA and Both miRNA Mimic and miRNA Inhibitor in 24-Well Plates

This protocol is provided as a starting point for optimization of miRNA mimic, miRNA inhibitor, and plasmid DNA cotransfection in HeLa S3 cells using Attractene Transfection Reagent. The amounts given are for one well of a 24-well plate. In this protocol, cell seeding and transfection are performed on the same day. For use with other adherent cell types, optimization of the protocol parameters will be necessary.

Important point 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.

Things to do before starting

- If you have ordered miRNA mimics or inhibitors from QIAGEN, they are delivered lyophilized and must be resuspended prior to transfection. To resuspend, follow the instructions provided on page 13.

Procedure

1. **Shortly before transfection, dilute 6 x 10^4 cells in 500 µl 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 prepared after step 4 of this protocol.
3. **Dilute 400 ng plasmid DNA and 0.14 µl miRNA mimic (20 µM stock) in 30 µl medium without serum or antibiotics (this will give a final miRNA mimic concentration of 5 nM after adding cells to complexes in step 7). Add 0.75 µl Attractene Transfection Reagent. Mix by pipetting up and down.**

   Note: Depending on the reporter system, it may be necessary to reduce the amount of miRNA mimic used. For reporters that respond strongly to the miRNA mimic, we recommend starting with 0.69 ng miRNA mimic (this will give a final miRNA mimic concentration of 0.1 nM after adding cells to complexes in step 7).

   IMPORTANT: The amount of Attractene Transfection Reagent, plasmid DNA, and miRNA mimic required for optimal performance may vary, depending on the detection system.

4. **Dilute 1.4 µl miRNA inhibitor (20 µM stock) in 30 µl medium without serum or antibiotics (this will give a final miRNA inhibitor concentration of 50 nM after adding cells to complexes in step 7). Add 0.75 µl Attractene Transfection Reagent. Mix by pipetting up and down.**

   IMPORTANT: The amount of Attractene Transfection Reagent and miRNA inhibitor required for optimal performance may vary, depending on the detection system.

5. **Incubate for 10–15 min at room temperature (15–25°C) to allow the formation of transfection complexes.**
6. **Mix the miRNA mimic–DNA complexes from step 3 and the miRNA inhibitor complexes from step 4. Then add the complexes to a single well of a 24-well plate.**
7. **Add the 500 µl cell culture from step 1 to the well. Gently swirl the plate to ensure uniform distribution of the cells.**
8. **Incubate the cells with the transfection complexes under their normal growth conditions and monitor gene expression after an appropriate time (e.g., 24–72 h after transfection, depending on experimental setup). Change the medium as required.**

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

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 after transfection of miRNA mimic/inhibitor	
a) Suboptimal HiPerFect Transfection Reagent:miRNA mimic/inhibitor ratio	Although fixed volumes of HiPerFect Transfection Reagent usually work very well with a range of miRNA mimic/inhibitor 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. To optimize the HiPerFect Transfection Reagent to miRNA mimic/inhibitor ratio, perform systematic titrations of HiPerFect Transfection Reagent (for more information on optimization, consult the *HiPerFect Transfection Reagent Handbook* at www.qiagen.com/HB/HiPerFect).
b) Suboptimal cell density	If cell density at the time of addition of HiPerFect Transfection Reagent–miRNA mimic/inhibitor 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 miRNA mimic/inhibitor.
Excessive cell death	
a) Concentration of transfection Reagent–miRNA mimic/inhibitor complexes too high	Decrease the amount of transfection reagent–miRNA mimic/inhibitor complexes added to the cells.

	Comments and suggestions
b) Cells stressed	Avoid stressing cells with temperature shifts and long periods without medium during washing steps. It is particularly important that the cells are in good condition for efficient transfection of miRNA mimic/inhibitor. Therefore, ensure that cell density is not too low at transfection.
c) Key gene silenced or key miRNA inhibited	If the gene/miRNA targeted is important for the survival of the cell, silencing/inhibiting this gene/miRNA may lead to cell death.
d) Poor DNA quality when using a reporter vector	DNA should be of high quality, as impurities can lower transfection efficiency. Plasmid DNA should be purified using HiSpeed®, QIAfilter, or QIAGEN Plasmid Kits. For endotoxin-sensitive cells, we recommend using EndoFree® Plasmid Kits (see www.qiagen.com/DNA).

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 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 miRNA mimic/inhibitor too low	Increase the concentration of miRNA mimic/inhibitor used for transfection.

Comments and suggestions	
No or very small gene silencing effect after miRNA mimic transfection	
a) Incubation time after transfection too short	The gene silencing effect observed at the protein level is dependent on the expression level of the protein and its rate of turnover within the cell. Perform a time-course experiment to determine the optimal time point for analysis.
b) Concentration of miRNA mimic too low	Increase the concentration of miRNA mimic used for transfection.
c) Inappropriate experimental approach	For many targets, miRNA effects cannot be detected at the transcript level. If possible, repeat experiments using a different experimental approach (e.g., if currently detecting transcript levels using PCR, try to detect protein levels instead). If possible, include both positive and negative controls in your experiments.
d) miRNA has only modulatory function	miRNA regulatory effects may be less obvious than gene silencing by siRNA. miRNAs can also have a modulatory function in which they regulate gene expression without completely silencing the gene. Several miRNAs may have different regulatory effects on the same target. If possible, select a different target (as most miRNAs have a large variety of targets) or use a different miRNA mimic for the same target.
e) Target under study not regulated by chosen miRNA	miRNAs are naturally occurring noncoding RNAs with binding patterns that differ from those of siRNAs. This makes target prediction difficult and a high score on available target prediction software does not guarantee that the investigated target is regulated by the miRNA of interest. If possible, include both positive and negative controls in your experiments.
f) Multiple target transcripts exist with different 3' UTRs	miRNA regulates translational repression by binding to the 3' UTR of the target mRNA. Many cellular proteins are translated from 2 or more different transcripts. Often the 3' UTRs of these transcripts differ significantly, with one transcript having a specific miRNA binding site in the 3' UTR, while the other does not.

Comments and suggestions	
No or very small inhibitory effect after miRNA inhibitor transfection	
a) Incubation time after transfection too short	The inhibitory effect observed at the protein level is dependent on the rate of target protein synthesis. Perform a time-course experiment to determine the optimal time point for analysis.
b) Concentration of miRNA inhibitor too low	Increase the concentration of miRNA inhibitor used for transfection.
c) Inappropriate experimental approach	For many targets, miRNA effects cannot be detected at the transcript level. If possible, repeat experiments using a different experimental approach (e.g., if currently detecting transcript levels using PCR, try to detect protein levels instead). If possible, include both positive and negative controls in your experiments.
d) Target miRNA has only modulatory function	miRNA regulatory effects may be less obvious than gene silencing by siRNA. miRNAs can also have a modulatory function in which they regulate gene expression without completely silencing the gene. For this reason, inhibiting a specific miRNA may not lead to significant changes at the transcript or protein level.
e) More than one miRNA regulates the target	Many targets are regulated by more than one miRNA; therefore inhibiting one miRNA may not abolish translational repression by the other miRNAs targeting the same gene. If possible, use a cell system with only a few of the endogenous miRNAs that target your gene of interest. Alternatively, select a target that is regulated by only a few endogenous miRNAs.
f) Target under study is not regulated by the chosen miRNA	miRNAs are naturally occurring noncoding RNAs with binding patterns that differ from those of siRNAs. This makes target prediction difficult and a high score on available target prediction software does not guarantee that the investigated target is regulated by the miRNA of interest. If possible, include both positive and negative controls in your experiments.

	Comments and suggestions
Low reporter expression from an unregulated-vector control	
a) Suboptimal Attractene Reagent to DNA ratio	If the ratio of transfection reagent to plasmid DNA is suboptimal, the overall charge of the complexes may be negative, neutral, or strongly positive, which can lead to inefficient adsorption to the cell surface. Optimize the Attractene Transfection Reagent to DNA ratio using Table 4, page 16.
b) Insufficient Attractene Reagent–DNA complex	If the transfection efficiency is lower than expected and cytotoxicity is acceptably low, increase the overall amount of Attractene Reagent–DNA complex added to the cells
c) Suboptimal incubation time	Different cell types achieve maximal expression levels at different times after transfection. This should be kept in mind when determining the length of incubation after transfection. If the time point of maximal expression is not known for a particular cell line, a time-course experiment may be necessary.
d) Suboptimal cell density	If cell density at the time of adding Attractene Reagent–DNA complexes is not at an optimal level, this can lead to insufficient uptake of complexes into the cells.
e) Vector influence	Factors such as the promoter, origin of replication, and plasmid size influence gene expression rate. The optimal quantity of plasmid DNA used for transfection is dependent on the expression rate of the plasmid.
f) Poor DNA quality	DNA should be of high quality, as impurities can lower transfection efficiency. Plasmid DNA should be purified using HiSpeed, QIAfilter, or QIAGEN Plasmid Kits. For endotoxin-sensitive cells, we recommend using EndoFree Plasmid Kits.

Appendix A: General Remarks on Handling RNA

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to degrade RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and nondisposable vessels and solutions while working with RNA.

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 or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible.

Disposable plasticware

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

Nondisposable plasticware

Nondisposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH,* 1 mM EDTA* followed by RNase-free water (see "Solutions", page 34). Alternatively, chloroform-resistant plasticware can be rinsed with chloroform* to inactivate RNases.

* 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.

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent,* thoroughly rinsed, and oven baked at 240°C for 4 hours or more (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate). Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight (12 hours) at 37°C, and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.

Solutions

Solutions (water and other solutions) should be treated with 0.1% DEPC. DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Add 0.1 ml DEPC to 100 ml of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris* buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO_2. When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

Note: QIAGEN solutions, such as RNase-free water, are guaranteed RNase-free without using DEPC treatment and are therefore free of any DEPC contamination.

* 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.

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.
miScript miRNA Mimic (1 nmol or 5 nmol)	1 nmol (for approx. 400 transfections in 24-well plates)* or 5 nmol (for approx. 2000 transfections in 24-well plates)* cell-culture–grade miRNA mimic, provided in tubes	Varies†
miScript miRNA Mimic (20 nmol)	20 nmol animal-grade (HPLC-purified) miRNA mimic, provided in tubes	Varies†
miScript miRNA Inhibitor (1 nmol or 5 nmol)	1 nmol (for approx. 40 transfections in 24-well plates)‡ or 5 nmol (for approx. 200 transfections in 24-well plates)‡ cell-culture–grade miRNA inhibitor, provided in tubes	Varies†
miScript miRNA Inhibitor (20 nmol)	20 nmol animal-grade (HPLC-purified) miRNA inhibitor, provided in tubes	Varies†
miScript miRNA Inhibitor (ps, 20 nmol)	20 nmol animal-grade (HPLC-purified) miRNA inhibitor, phosphorothioate modified for in vivo studies, provided in tubes	Varies†
HiPerFect Transfection Reagent (0.5 ml)	Transfection reagent for efficient miRNA mimic or inhibitor transfection	301704
Attractene Transfection Reagent (0.5 ml)§	Transfection reagent for efficient cotransfection of vector DNA and miRNA mimic and/or inhibitor	301004
For positive control experiments		
Syn-hsa-miR-1 miScript miRNA Mimic	1 nmol, 5 nmol, or 20 nmol hsa-miR-1 mimic, provided in tubes	MSY0000416

* Refers to 5 nM final mimic concentration in a 24-well plate with a final volume of 500 µl.

† Visit www.qiagen.com/GeneGlobe/miRNAproducts to search for and order these products; for custom design (for miRNAs not listed in miRBase), visit www.qiagen.com/miDesign.

‡ Refers to 50 nM final inhibitor concentration in a 24-well plate with a final volume of 500 µl.

§ Various sizes available; please inquire.

Product	Contents	Cat. no.
Anti-hsa-miR-1 miScript miRNA Inhibitor	1 nmol, 5 nmol, or 20 nmol hsa-miR-1 inhibitor, provided in tubes; option of phosphorothioate modification at 20 nmol scale	MIN0000416
For negative control experiments		
AllStars Negative Control siRNA (5 nmol)	Validated siRNA with no homology to any known mammalian gene, for use as a nonsilencing control in miRNA/siRNA experiments	1027280
miScript Inhibitor Negative Control	5 nmol cell-culture–grade or 20 nmol animal-grade negative control miRNA inhibitor, provided in tubes	Inquire
For transfection control experiments		
AllStars Hs Cell Death Control siRNA (5 nmol)	Positive cell-death phenotype control	1027298
For miRNA purification		
miRNeasy Mini Kit (50)	For 50 preps: 50 RNeasy® Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), QIAzol® Lysis Reagent, RNase-Free Reagents and Buffers	217004
miRNeasy 96 Kit (4)	For 4 x 96 preps: 4 RNeasy 96 plates, Collection Microtubes (racked), Elution Microtubes CL, Caps, S-Blocks, AirPore Tape Sheets, QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	217061
miRNeasy FFPE Kit (50)	For 50 preps: 50 RNeasy MinElute® Spin Columns, 50 gDNA Eliminator Spin Columns, Collection Tubes, Proteinase K, RNase-Free Reagents and Buffers	217404
For miRNA detection by real-time PCR		
miScript Reverse Transcription Kit (10)	For 10 reactions: miScript Reverse Transcriptase Mix, miScript RT Buffer, RNase-Free Water	218060

Product	Contents	Cat. no.
miScript Reverse Transcription Kit (50)	For 50 reactions: miScript Reverse Transcriptase Mix, miScript RT Buffer, RNase-Free Water	218061
miScript SYBR® Green PCR Kit (200)	For 200 reactions: QuantiTect SYBR Green PCR Master Mix, miScript Universal Primer	218073
miScript SYBR Green PCR Kit (1000)	For 1000 reactions: QuantiTect SYBR Green PCR Master Mix, miScript Universal Primer	218075
miScript Primer Assay (100)	10x miScript Primer Assay (contains one miRNA-specific primer)	Varies*
Human miScript Assay 96 Set (100 or 20)†	714 miScript Primer Assays targeting human miRNAs provided in 96-well plates; for 100 x 50 µl reactions or 20 x 50 µl reactions	Varies
Mouse miScript Assay 96 Set (100 or 20)†	561 miScript Primer Assays targeting mouse miRNAs provided in 96-well plates; for 100 x 50 µl reactions or 20 x 50 µl reactions	Varies
Rat miScript Assay 96 Set (100 or 20)†	346 miScript Primer Assays targeting rat miRNAs provided in 96-well plates; for 100 x 50 µl reactions or 20 x 50 µl reactions	Varies
miScript Primer Assay 96 Plate (20 or 100)	miRNA-specific primers provided in 96-well plates; 20 or 100 reactions; minimum order 24 assays	Varies‡
miScript Primer Assay 384 Plate (20)	miRNA-specific primers provided in 384-well plates; 20 reactions; minimum order 96 assays	Varies‡

* Visit www.qiagen.com/GeneGlobe to search for and order these products; for custom design (for miRNAs not listed in miRBase), visit www.qiagen.com/miDesign.

† Also available in 384-well plates for 20 reactions.

‡ Visit www.qiagen.com/miPlate to order miScript Plates.

Product	Contents	Cat. no.
miScript miRNA Mimics and Inhibitors on plates		
miScript miRNA Mimic 96 Plate (1 nmol or 5 nmol)	1 nmol or 5 nmol cell-culture–grade miRNA mimics in 96-well plates; minimum order 24 mimics	Varies*
miScript miRNA Mimic 384 Plate (1 nmol)	1 nmol cell-culture–grade miRNA mimics in 384-well plates; minimum order 96 mimics	Varies*
miScript Inhibitor 96 Plate (1 nmol or 5 nmol)	1 nmol or 5 nmol cell-culture–grade miRNA inhibitors in 96-well plates; minimum order 24 inhibitors	Varies*
miScript Inhibitor 384 Plate (1 nmol)	1 nmol cell-culture–grade miRNA inhibitors in 384-well plates; minimum order 96 inhibitors	Varies*
For plasmid DNA purification		
HiSpeed Plasmid Midi Kit (25)	25 HiSpeed Midi Tips, 25 QIAfilter Midi Cartridges, 25 QIAprecipitator Midi Modules plus Syringes, Reagents, Buffers	12643
QIAfilter Plasmid Midi Kit (25)†	25 QIAGEN-tip 100, Reagents, Buffers, 25 QIAfilter Midi Cartridges	12243
QIAGEN Plasmid Mini Kit (25)†	25 QIAGEN-tip 20, Reagents, Buffers	12123
EndoFree Plasmid Maxi Kit (10)	10 QIAGEN-tip 500, Reagents, 10 QIAfilter Maxi Cartridges, Endotoxin-Free Buffers	12362

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* Visit www.qiagen.com/miPlate to order miScript Plates.

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

Notes

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Austria ■ Orders 0800/28-10-10 ■ Fax 0800/28-10-19 ■ Technical 0800/28-10-11

Belgium ■ Orders 0800-79612 ■ Fax 0800-79611 ■ Technical 0800-79556

Canada ■ Orders 800-572-9613 ■ Fax 800-713-5951 ■ Technical 800-DNA-PREP (800-362-7737)

China ■ Orders 0086 21 3865 3865 ■ Fax 0086 21 3865 3965 ■ Technical 800 988 0325, 800 988 0327

Denmark ■ Orders 80-885945 ■ Fax 80-885944 ■ Technical 80-885942

Finland ■ Orders 0800-914416 ■ Fax 0800-914415 ■ Technical 0800-914413

France ■ Orders 01-60-920-926 ■ Fax 01-60-920-925 ■ Technical 01-60-920-930 ■ Offers 01-60-920-928

Germany ■ Orders 02103-29-12000 ■ Fax 02103-29-22000 ■ Technical 02103-29-12400

Hong Kong ■ Orders 800 933 965 ■ Fax 800 930 439 ■ Technical 800 930 425

Ireland ■ Orders 1800-555-049 ■ Fax 1800-555-048 ■ Technical 1800-555-061

Italy ■ Orders 02-33430411 ■ Fax 02-33430426 ■ Technical 800-787980

Japan ■ Telephone 03-5547-0811 ■ Fax 03-5547-0818 ■ Technical 03-5547-0811

Korea (South) ■ Orders 1544 7145 ■ Fax 1544 7146 ■ Technical 1544 7145

Luxembourg ■ Orders 8002-2076 ■ Fax 8002-2073 ■ Technical 8002-2067

The Netherlands ■ Orders 0800-0229592 ■ Fax 0800-0229593 ■ Technical 0800-0229602

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