Flexible RNAi Technologies You Can Rely On

QIAGEN®

Sample & Assay Technologies

Introduction

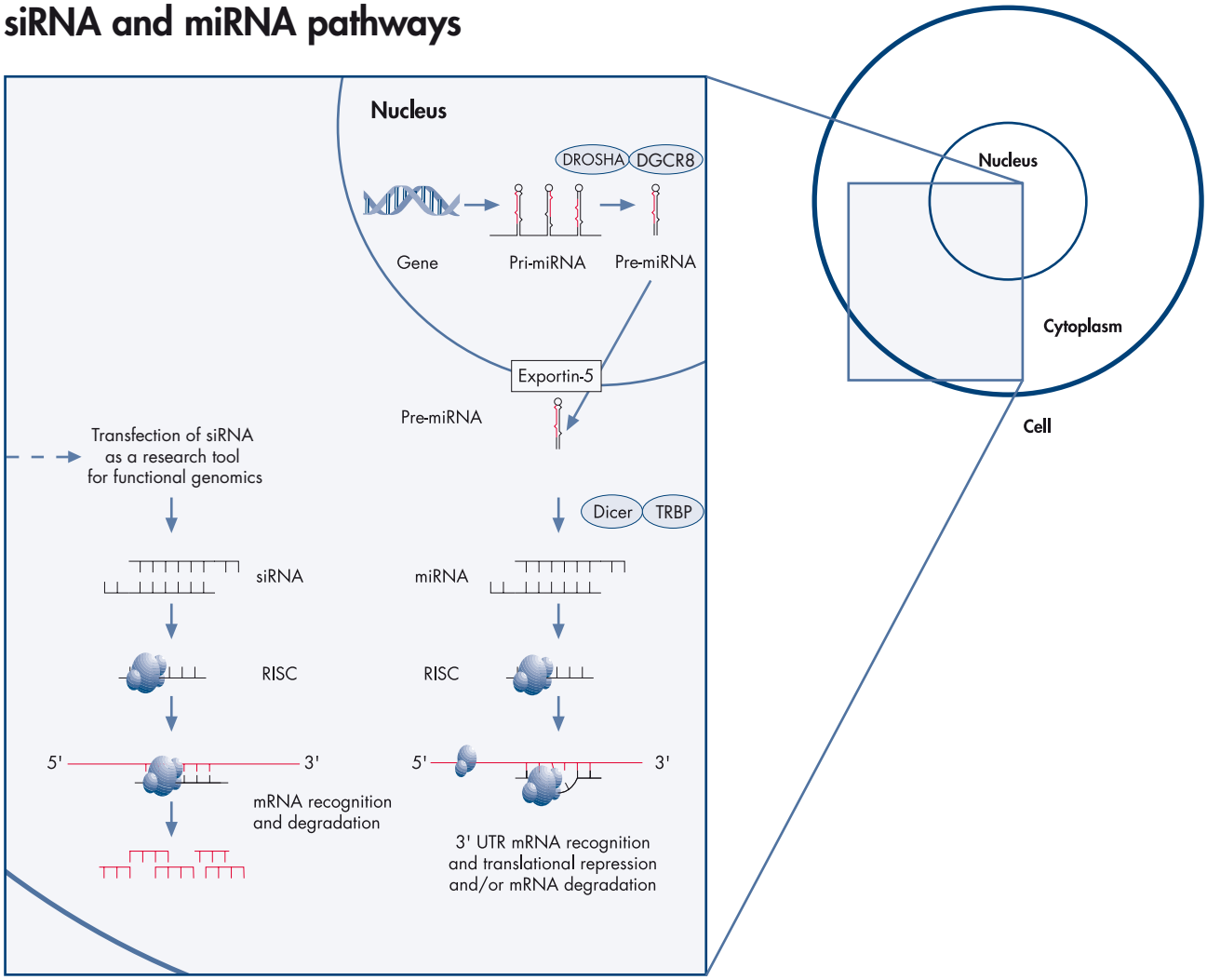
RNAi is an invaluable tool for functional genomics and drug discovery research. In this brochure, you can discover QIAGEN's RNAi product portfolio, consult recommendations for control experiments, and read about critical factors. Whatever your throughput level, QIAGEN provides flexible, innovative technologies to facilitate success in your research!

Resources

GeneGlobe Web portal: source of gene- and pathway-specific products
www.qiagen.com/GeneGlobe

- RNAi products and resources
  www.qiagen.com/siRNA
- TransFect Protocol Database for cell-type specific transfection protocols
  www.qiagen.com/TransFect
- Transfection Cell Database: a comprehensive list of transfected cell lines with experimental details
  www.qiagen.com/TransfectionCellDatabase
- QIAGEN AllStars RNAi Controls page: information about appropriate RNAi controls
  www.qiagen.com/AllStars
- Literature, journal references, tips and tools, and more
  www.qiagen.com/Support
- QIAGEN ProductFinder
  www.qiagen.com/ProductFinder

siRNA and miRNA pathways



In functional genomics research, chemically synthesized short interfering RNAs (siRNAs) are transfected into cells. An RNA-induced silencing complex (RISC) is assembled, siRNAs unwind, and a single strand of RNA remains bound to the RISC. The RISC targets mRNA transcripts that have complementary sequences to the bound RNA and cleaves the homologous mRNA, preventing translation.

miRNAs are first transcribed in the nucleus as long, primary miRNAs (pri-miRNA) which are then processed into precursor miRNAs (pre-miRNA) by the nuclear microprocessor complex (Drosha and DGCR8). Pre-miRNAs are transported into the cytosol by Exportin-5. Dicer and TRBP process the pre-miRNAs into mature miRNAs that are incorporated into the RISC. When the RISC identifies the complementary or partially complementary mRNA target, it inhibits gene expression by translational repression or by mRNA degradation.

siRNA	miRNA
Exogenous siRNA used as a research tool	Endogenous
Perfect complementarity to target mRNA	Often partial complementarity to target mRNA
Sequence-specific target mRNA degradation	Translational repression and/or target mRNA degradation

Critical parameters for successful RNAi

Planning an RNAi experiment begins with a biological question or hypothesis, and this helps to define many of the parameters incorporated into the experimental design. These parameters include the type and number of gene targets, the choice of cell line and method of siRNA delivery, the downstream assay, appropriate controls (see page 8), time points for analysis, and finally, the statistical methodology for data analysis.

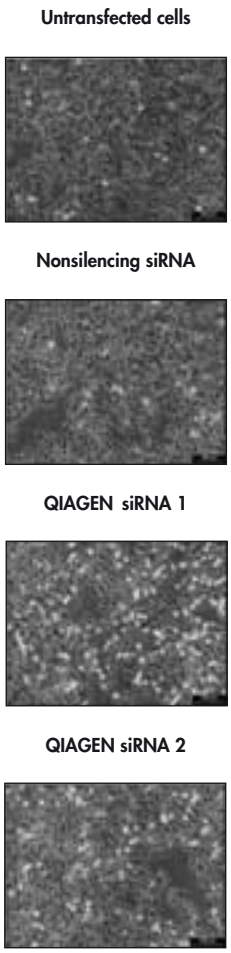


Figure 1. Confirmation of phenotype with 2 siRNAs. A549 cells were examined 24 hours after transfection with PLK1 siRNAs or nonsilencing siRNA. PLK1 knockdown characteristically results in the accumulation of rounded, apparently mitotic cells. This phenotype is clearly visible after transfection of each of the 2 siRNAs designed by QIAGEN. (Data kindly provided by Ralph Graeser, Sarah Umber, Michaela Brosig, and Michael H.G. Kubbutat, ProQinase GmbH, Freiburg, Germany.)

Gene and siRNA selection

RNAi experiments range in scale from the knockdown of a single gene of interest, to small numbers of related genes or genes within a common pathway, to genomewide high-throughput screens. In addition to individual siRNAs in tubes, QIAGEN provides FlexiPlate siRNA, which allows you to pick and choose gene targets for medium- to high- throughput experiments and to custom array the siRNAs in plates (page 13). QIAGEN has also developed and validated several control siRNAs which should be included in each RNAi experiment to ensure reliable interpretation of results (page 15).

Optimized transfection

The cell type chosen for RNAi experiments should allow efficient siRNA delivery. Transfection must be optimized to ensure that siRNA is transfected at the lowest effective concentration to avoid unwanted nonspecific effects. The transfection method selected should provide high efficiency and low cytotoxicity, and should be amenable to the experimental setup, for example, suitable for reverse transfection in an automated format.

Robust phenotypic analysis

Phenotypic analysis often involves cell-based assays, which are designed to measure a variety of cellular parameters, including viability, production of detectable products, changes in protein localization, ion concentration, cellular motility, and morphology. Characterization of changes in protein localization and protein modification can provide important clues about gene function (see Qproteome® Kits, page 18). Cell-based assays measuring pathway activity can reveal information about gene function and regulation (see Cignal™ Reporter System at www.SABiosciences.com).

Knockdown verification

QIAGEN recommends using at least 2 siRNAs that target different regions within the same transcript. The probability that the same off-target phenotype is induced by 2 independent siRNAs for the same target gene is extremely low. Therefore, confirming a phenotype with distinct siRNAs is an easily applied and convincing way to verify that the observed phenotype is specific to the target gene knockdown (Figure 1). Confirmation with multiple siRNAs has become a requirement for publication of RNAi results in many peer-reviewed journals. Knockdown of the target gene can be verified at the mRNA level by real-time RT-PCR or at the protein level by western blotting (page 18). In addition to confirmation with multiple siRNAs, a rescue experiment can be performed to provide conclusive evidence that knockdown of a targeted gene, and not an off-target effect, is responsible for an observed phenotype (page 8).

RNAi workflow

siRNA selection

- FlexiTube siRNA
  FlexiTube siRNA Premix
  FlexiPlate siRNA
- SureSilencing™ shRNA Plasmids
- Biology-on-Array™ Systems
- QIAGEN AllStars
  RNAi Controls

siRNA transfection

- HiPerFect® Transfection
  Reagent

Protein purification

- Qproteome Kits

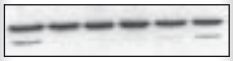
RNA purification

- RNeasy® Kits
- AllPrep® Kits
- miRNeasy Kits

Phenotypic assay

- Cignal
  Reporter
  System

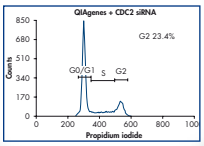
Western blot



cDNA preparation

- QuantiTect® Reverse
  Transcription Kit
- FastLane® Kits

Rescue experiment



- Rescue
  siRNA
- QIAgenes
  Expression
  Kits

Real-time PCR analysis

- Rotor-Gene® Q cycler
  and Rotor-Gene Kits
- QuantiTect Primer Assays
  QuantiTect and QuantiFast®
  RT-PCR and PCR Kits
- miScript PCR System
- Pathway-Powered PCR Arrays

QIAGEN's sample and assay technologies enable seamless integration of every step of your RNAi workflow.

siRNA design

siRNAs from QIAGEN are designed using cutting-edge HP OnGuard siRNA Design, which delivers potent and specific siRNA. Multiple siRNAs for every human, mouse, and rat gene in the EntrezGene database can be ordered at the QIAGEN GeneGlobe Web portal (page 9). HP OnGuard siRNA Design incorporates many unique and advanced features (Table 1).

Table 1. HP OnGuard siRNA Design features

Feature	Description	Benefit	References
Neural-network technology	siRNA design uses the BioPredsi neural network which is based on an extremely large RNAi data set.	Potent siRNA	1–3
The world's largest siRNA validation project	Data from this project, in which QIAGEN scientists proved the effectiveness of thousands of siRNAs, were used to reinforce and improve the design process.	A large number of druggable genome siRNAs have been proven in wet-lab experiments to provide at least 70% knockdown during this project; insights from the project enhanced design of potent siRNA	4
Homology analysis	A proprietary tool and an up-to-date, nonredundant sequence database are used.	Decreases off-target effects	
Affymetrix® GeneChip® analysis	Genomewide analysis enabled development of siRNA design improvements that minimize off-target effects.	Insights from the analysis enhanced design of specific siRNA	
Up-to-date siRNA target sequences	Current data from NCBI databases ensure accurate design.	Accurate siRNA design	
Asymmetry	siRNAs are designed with unequal stabilities of the base pairs at the 5' end of the antisense strand. This enables the antisense strand, which is less tightly bound at its 5' end, to enter RISC, while the sense strand is degraded.	Produces highly functional siRNAs and reduces the risk of off-target effects caused by the incorrect strand entering RISC	5, 6
3' UTR/seed region analysis	Intelligently weighted, multi-parameter searches for matches of the seed region of the siRNA antisense strand with the 3' untranslated region of unintended mRNA targets are performed.	Minimizes risk of off-target effects caused by siRNA acting like an miRNA	7–12
SNP avoidance	The RefSNP database is used to exclude siRNAs which span single nucleotide polymorphisms (SNPs).	Increases siRNA potency, as an siRNA spanning a SNP will vary in its effectiveness	
Interferon motif avoidance	siRNAs are screened for multiple sequence motifs known to result in an interferon response. siRNAs with such motifs are rejected.	Reduces risk of off-target effects caused by an interferon response	13, 14

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siRNA delivery — key questions and answers

How can I optimize siRNA transfection?

siRNA transfection should be optimized when establishing RNAi in the lab and for every new cell line studied. The *HiPerFect Transfection Reagent Handbook* contains useful information on optimization (www.qiagen.com/HB/HiPerFect). In optimization experiments, transfection efficiency can be monitored by transfection of a positive control, such as AllStars Cell Death Control siRNA (page 15).

Transfection factors that should be optimized include:

- Reagent and siRNA amounts
- Cell confluency at transfection
- Incubation time of cells and complexes prior to analysis

Why is it important to transfect low siRNA amounts?

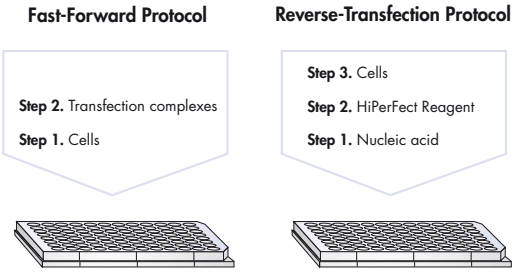
Transfection of siRNA at high concentrations can result in off-target effects, in which siRNAs affect the expression of partially homologous or nonhomologous genes. Research suggests that off-target effects, which may produce misleading results in RNAi experiments, can be largely avoided by using low siRNA concentrations (1, 2). HiPerFect Transfection Reagent facilitates highly effective siRNA uptake, which enables gene silencing using low siRNA concentrations without compromising knockdown efficiency.

Why should cytotoxicity be avoided during siRNA transfection?

Cytotoxicity caused by siRNA delivery interferes with data analysis after RNAi due to unreliable phenotypic analysis. Factors affecting data analysis include changes in phenotype caused by the transfection reagent, changes in gene expression caused by stress, and the presence of dead cells which should not be used for analysis. HiPerFect Transfection Reagent is not toxic to cells and does not cause vacuole formation (which indicates disruption of cellular processes). Detailed cell cycle and cell morphology analyses show no differences between mock-transfected cells (HiPerFect Transfection Reagent alone) and untransfected cells.

What is the difference between forward and reverse transfection?

In reverse-transfection protocols, siRNA is added to plate wells first, followed by transfection reagent. After complex formation, cells are added. This is in contrast to forward transfection, where cells are added to plate wells first, and complexes are added afterwards. Reverse transfection is widely used, especially for high-throughput experiments, because it is easily automatable, siRNAs can be stored in plate wells prior to transfection, and less material is used as it is not necessary to perform siRNA–transfection reagent complex formation in a separate plate.



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RNAi controls

It is important to perform suitable control experiments to ensure that results can be correctly interpreted (see also page 15).

Positive and transfection control siRNA

A positive control siRNA is known to provide high knockdown of its target mRNA. A positive control is used to establish that the experimental setup for transfection and knockdown analysis is working optimally. An siRNA that knocks down a gene resulting in the phenotypic effect under study may be used as a positive control. QIAGEN provides thousands of validated siRNAs for human genes that could serve as positive controls. Alternatively, siRNA that induces a detectable phenotype, such as the AllStars Cell Death Control siRNAs (page 15), can be used for this purpose. A positive control siRNA should be transfected in every experiment.

Negative control siRNA

A negative control siRNA is typically an siRNA with no homology to any known mammalian gene. It should also be tested to ensure that it does not affect cell phenotype (see AllStars Negative Control siRNA, page 15). Transfection of negative control siRNA establishes a baseline of effect, which is used to determine whether changes in phenotype or gene expression are nonspecific. A negative control siRNA should be transfected in every experiment.

Mock-transfection control

Mock-transfected cells go through the transfection process without addition of siRNA (i.e., cells are treated with transfection reagent only). This control is used to determine any nonspecific effects that may be caused by the transfection reagent or process.

Untransfected cells control

Gene expression analysis should be carried out on cells that have not been treated to allow measurement of the normal, 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.

Phenotype confirmation using multiple siRNAs

A phenotypic effect caused by knockdown of a gene must be confirmed using at least one additional siRNA targeted against a different area of the mRNA.

Rescue experiments

Rescue experiments can be performed to provide conclusive evidence that knockdown of a targeted gene, and not an off-target effect, is responsible for an observed phenotype. In a typical rescue experiment, cells that display an altered phenotype after siRNA-mediated target gene knockdown are shown to display the wild-type phenotype after cotransfection of siRNA and a construct expressing a variant of the target gene that is not regulated by the siRNA. The gene expressed by the construct compensates for the endogenous gene knockdown. The GeneGlobe Web portal enables access to siRNAs and QIAgenes Expression Constructs for every human gene. QIAgenes Expression Constructs are ready-to-use expression vectors containing optimized protein-coding sequences for human genes. Rescue siRNAs target the 3' noncoding regions of the genes of interest. These noncoding sequences are not present in the QIAgenes Expression Construct. This allows knockdown of endogenous target genes with rescue siRNAs and exogenous expression of the same protein from siRNA-resistant constructs.

QIAGEN's GeneGlobe Web portal

GeneGlobe is an easy-to-use Web portal that that allows you to find information about, search for, and order gene- and pathway-specific products for multiple species. GeneGlobe provides access to more than 1.5 million products, facilitating RNAi, gene expression analysis, protein expression, and miRNA research. Products available at GeneGlobe include FlexiTube siRNA and FlexiTube GeneSolution (page 12), FlexiTube siRNA Premixes (page 16), QIAGEN AllStars RNAi Controls (page 15), QuantiTect Primer Assays (page 18), FlexiPlate siRNA (page 13), miScript Primer Assays, miScript miRNA Inhibitors and Mimics (www.qiagen.com/miRNA), and QIAgenes Expression Constructs (www.qiagen.com/goto/QIAgenes). Products are frequently updated to reflect the most up-to-date sequence information. Simply visit www.qiagen.com/GeneGlobe (Figure 2).

GeneGlobe provides:

- Easily accessible, ready-to-go solutions for RNAi
- Matching standardized siRNAs and real-time RT-PCR assays
- Interactive pathway information and resources

Figure 2. The search page at GeneGlobe. Products can be easily accessed by simply entering search terms, such as gene symbols or Entrez Gene IDs, at the GeneGlobe search page (www.qiagen.com/GeneGlobe).

Figure 3. GeneGlobe pathways. Pathway information is provided as easy-to-read graphics.

GeneGlobe pathways

GeneGlobe pathways provide valuable background information on gene families and pathways with easy-to-read graphics, enabling further elucidation of the role of target genes and planning of future experiments (Figure 3). Detailed descriptions and references are provided for each pathway. Products available for each protein in the pathway can be found by simply clicking on the protein image.

Knowledge resource

GeneGlobe is a useful resource for information about genes and pathways. Transcript maps clearly show the location of siRNAs, primer assays, and cDNAs of QIAgenes Expression Constructs (Figure 4). Links to NCBI Web pages provide easy access to comprehensive information about the gene of interest. Stem-loop images are provided for miRNA products.

siRNA information includes results of 3' UTR/seed region analysis performed during design (see also page 6). For experimentally validated siRNAs, valuable experimental information, such as the cell line used for validation, transfection conditions, and percentage knockdown achieved is provided. Rescue experiments are made easy using rescue siRNAs for every human gene that have been specifically designed for use with QIAgenes Expression Constructs (page 8). Recommended controls and related products are also easy to access.

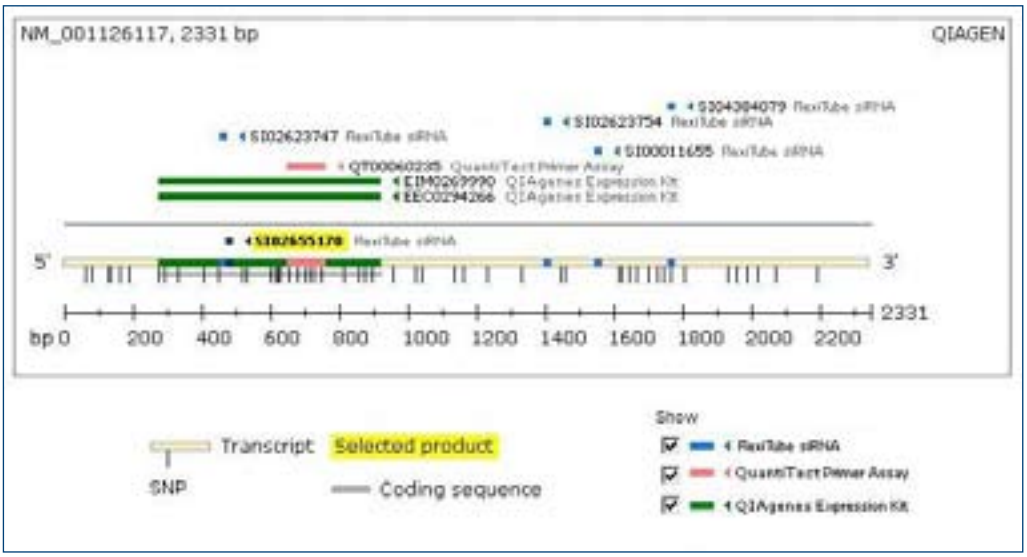
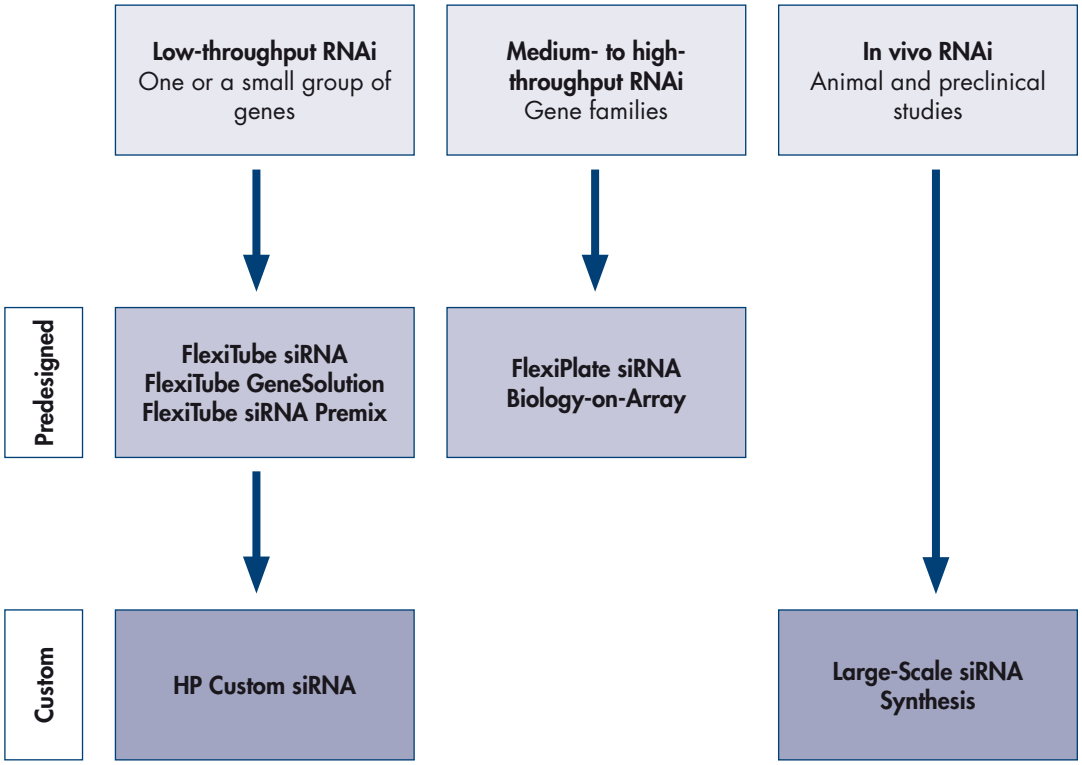


Figure 4. Transcript map at GeneGlobe.

RNAi selection guide



HiPerFect Transfection Reagent

QIAGEN AllStars RNAi Controls — a broad collection of extensively characterized controls for every aspect of RNAi experiments

New to RNAi? Choose the RNAi Human/Mouse Starter Kit — all you need for start-up and optimization experiments.

Table 2. Number of transfections from 1 nmol siRNA

Format	Number of transfections*
6-well plate	41
12-well plate	83
24-well plate	166
48-well plate	332
96-well plate	504

* The number of transfections depends on the culture volume and the final siRNA concentration. These figures are based on a final siRNA concentration of 10 nM.

FlexiTube siRNA and FlexiTube GeneSolution

siRNA in convenient 1 nmol, 5 nmol, and 20 nmol amounts

- Cost-effective siRNA enables analysis of more genes
- Optimal solutions for every gene
- Innovative design minimizes the risk of off-target effects
- Easy to search for and order siRNA at GeneGlobe
- Thousands of human siRNAs have been experimentally verified

FlexiTube siRNA enables RNAi analysis of small numbers of human, mouse, or rat genes. Human, mouse, and rat siRNAs are provided in 5 nmol or 20 nmol amounts. For human and mouse siRNAs, cost-effective 1 nmol amounts are also available (minimum order of 4 siRNAs). The 1 nmol scale is sufficient for multiple transfections (Table 2). siRNAs are provided lyophilized in tubes. siRNAs in 20 nmol amounts are available with labels, including Alexa Fluor®, fluorescein, rhodamine, Cy®3, and Cy5 dyes, or modification options such as amino linkers, thio linkers, and phosphate modifications. Full siRNA sequence information is provided.

4-siRNA package provides a gene-specific solution

FlexiTube GeneSolution is a gene-specific package of 4 preselected siRNAs (1 nmol scale) for a human or mouse target gene (Figure 5). FlexiTube GeneSolutions enable researchers to follow published guidelines which recommend redundancy experiments to ensure accurate reporting of results from RNAi experiments (1-3). Redundancy experiments use several distinct siRNAs targeting different areas of the same mRNA to rule out off-target effects.

Figure 5. FlexiTube GeneSolutions make selection of siRNAs easy. Simply enter details of the human or mouse genes of interest at GeneGlobe, and the FlexiTube GeneSolution will be displayed.

References

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FlexiPlate siRNA

Sets for highly flexible, economical RNAi screening or screening follow-up

- Maximum flexibility to select siRNAs, controls, scales, and plate layout
- Economical scales (0.1 nmol, 0.25 nmol, 1 nmol) allow screening of more genes
- Fast and easy access via GeneGlobe
- Cutting-edge siRNA design minimizes the risk of off-target effects
- Thousands of human siRNAs have been experimentally verified

FlexiPlate siRNA provides completely flexible RNAi screening. siRNAs for any human or mouse target genes can be ordered at 0.1 nmol, 0.25 nmol, and 1 nmol scales in 96-well plates and in 0.1 nmol and 0.25 nmol scales in 384-well plates. Positive or negative controls can be selected from a wide range. For maximum flexibility, siRNAs can be selected and plate layout arranged as desired at the GeneGlobe Web portal (Figure 6).

GeneGlobe pathways (see page 10 and www.qiagen.com/GeneGlobe/Pathways) can be used to generate lists of siRNAs related to the same gene pathway. These lists can be used as a starting point for FlexiPlate siRNA from which siRNAs can be added, deleted, rearranged, and ordered in the scale of choice.

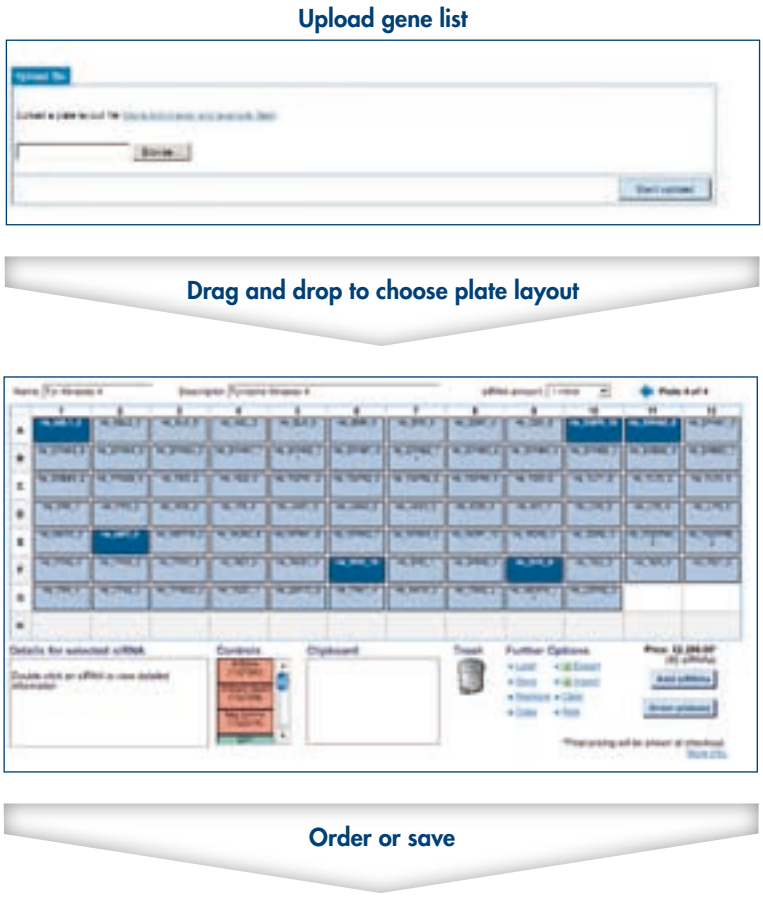

Figure 6. FlexiPlate siRNA creation and order process.

HP Custom siRNA

Custom synthesis of highly pure siRNA

- >90% purity from patented TOM amidite chemistry and purification processes
- Highly photostable and bright Alexa Fluor labels available
- Option of a range of modifications

HP Custom siRNA is an siRNA synthesis option that provides for specific siRNA requirements, including siRNA for multiple species, specific splice variants, and non-human, -mouse, and -rat genes. siRNAs are synthesized according to customer specifications and provided in tubes or 96-well plates. Modification options include Alexa Fluor, fluorescein, rhodamine, Cy3, and Cy5 dyes, as well as amino linkers, thio linkers, and phosphate modifications.

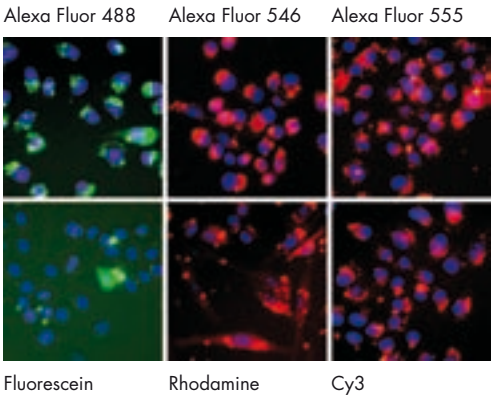

Figure 7. Alexa Fluor labeled siRNA provides brightest fluorescence. Fluorescence microscopy of HeLa S3 cells 24 hours after transfection with 100 nM siRNA labeled at the 3' end of the sense strand with different fluorescent dyes.

Large-Scale siRNA Synthesis

siRNA for in vivo animal studies and preclinical applications

- Flexibility to chose from a variety of scales and modifications
- High-purity siRNA with low endotoxin levels
- Reliable quality and expertise in large-scale synthesis

Large-Scale siRNA Synthesis from QIAGEN provides highly pure siRNA at a variety of scales ranging from 10 mg to 10 g for use in research and preclinical applications. Modification options include cholesterol, phosphate, amino hexyl, FITC, 3' OMe, 2' OMe, phosphorothioate, fluoropyrimidine, riboT, ribo inosine, biotin, and various dye labels. siRNA is purified by HPLC and is >90% pure. The availability of high-quality siRNA at large scales is essential for research into therapeutic uses of RNAi and in vivo animal studies.

QIAGEN AllStars RNAi Controls and the RNAi Human/Mouse Starter Kit

siRNAs, assays, and a kit for RNAi control experiments

- A broad range of extensively characterized RNAi controls
- Most thoroughly verified negative control siRNA available
- Kit comprises a complete set of controls to establish and optimize RNAi
- Cell death control enables straightforward transfection optimization

QIAGEN AllStars RNAi Controls are a comprehensive collection of the most extensively tested controls for every aspect of RNAi experiments in human, mouse, and rat (Table 3). AllStars Negative Control siRNA has been rigorously tested, using genomewide expression analysis and cell-based assays, and shown to provide minimal nonspecific effects. It has also been shown to enter RISC and is the most extensively verified negative control siRNA currently available. To view full data, visit www.qiagen.com/AllStars.

The RNAi Human/Mouse Starter Kit allows easy establishment of siRNA-mediated RNAi, optimization of transfection conditions, and routine control experiments. It includes AllStars Negative Control siRNA, MAPK1 positive control siRNA, AllStars Hs Cell Death Control siRNA, and HiPerFect Transfection Reagent. Additional tools from QIAGEN are available for validation of knockdown by western blot analysis (Tag·100 Antibody, cat. no. 34680) or quantitative, real-time RT-PCR (page 18).

Cell death controls enable easy transfection monitoring and positive control experiments

AllStars Hs Cell Death Control siRNA (for human) and AllStars Mm/Rn Cell Death Control siRNA (for mouse and rat) are blends of highly potent siRNAs targeting ubiquitously expressed genes that are essential for cell survival. Knockdown of these genes induces a high degree of cell death which is visible by light microscopy (Figure 8). Transfection efficiency can be quickly estimated by simply observing cells by straightforward light microscopy 48–96 hours after transfection of AllStars Cell Death Control siRNA, avoiding the need for any complex or laborious downstream assay.

Table 3. Range of RNAi controls

AllStars RNAi Controls	Description
AllStars Negative Control siRNA	Thoroughly tested nonsilencing siRNA
AllStars Cell Death Control siRNAs	Cell-death phenotype controls for transfection
AllStars Transfection Controls	Fluorescently labeled siRNAs and cell-death phenotype controls for monitoring transfection efficiency
AllStars Positive Controls	Routine positive controls
AllStars Reporter Controls	siRNAs targeting reporter assay genes
AllStars Interferon Controls	Real-time RT-PCR assays for detection of interferon-induced genes
AllStars Downstream Controls	Real-time RT-PCR assays for quantification of gene expression
RNAi Human/Mouse Starter Kit	Positive and negative controls, and HiPerFect Transfection Reagent

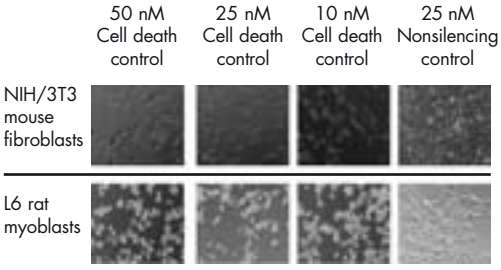

Figure 8. Fast and easy analysis of mouse and rat cells. NIH/3T3 or L6 cells (2 x 10^4/well) in 24-well plates were transfected with various amounts of AllStars Mm/Rn Cell Death Control siRNA or 25 nM nonsilencing siRNA (AllStars Negative Control siRNA) using HiPerFect Transfection Reagent. After 72 hours, cell death was observed by light microscopy. All conditions used induced a cell-death phenotype that could easily be distinguished from the nonsilencing control.

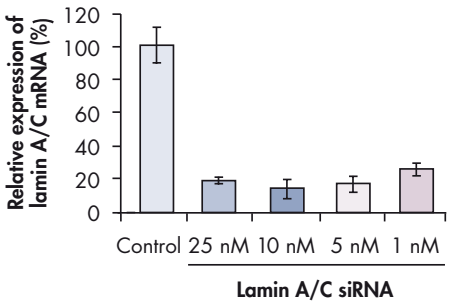

Figure 9. Successful transfection of primary cells. Normal Human Lung Fibroblasts were transfected with siRNA targeted against lamin A/C using HiPerFect Transfection Reagent. Nonsilencing siRNA was also transfected (**Control**). After 48 hours, knockdown was analyzed by quantitative, real-time RT-PCR.

HiPerFect Transfection Reagent

Unique reagent for exceptionally efficient knockdown using low siRNA concentrations

- Efficient transfection using low siRNA concentrations
- Effective transfection of primary cells with high cell viability
- Effective transfection of suspension cells and macrophages
- Efficient transfection of miRNA mimics or inhibitors

HiPerFect Transfection Reagent is a unique blend of cationic and neutral lipids that enables effective siRNA uptake and efficient release of siRNA inside cells, resulting in high gene knockdown even when using low siRNA concentrations (Figure 9). HiPerFect Transfection Reagent is highly suitable for high-throughput reverse transfection and also for miRNA research. A list of cell types that have been successfully transfected using HiPerFect Transfection Reagent and experimental details are available at www.qiagen.com/TransfectionCellDatabase. Transfection protocols for your cell type, nucleic acid, and format of interest are available at www.qiagen.com/TransFect (Figure 10).

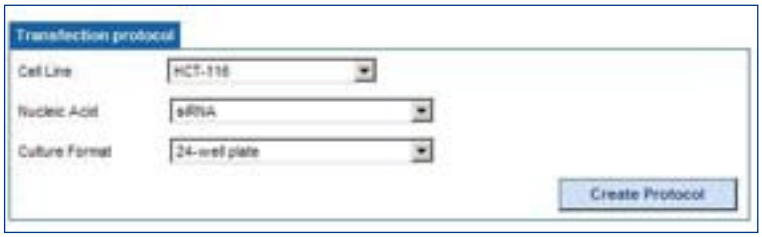

Figure 10. Easy-to-access transfection protocols. The TransFect Protocol Database provides a simple interface to access the protocol you need.

FlexiTube siRNA Premix

Ready-to-transfect, optimized siRNA–transfection reagent mix

- Highly convenient, ready-to-go transfection mix
- Minimal need for transfection optimization
- Efficient transfection with high cell viability

FlexiTube siRNA Premixes, available for every human and mouse gene, provide maximum convenience for gene silencing experiments. Premixed siRNA and transfection reagent saves time by eliminating mixing and complex formation protocol steps and eliminates the need for tedious optimization of siRNA:reagent ratio. FlexiTube siRNA Premix can be immediately transfected, allowing you to quickly achieve gene knockdown and focus on the results of your experiment.

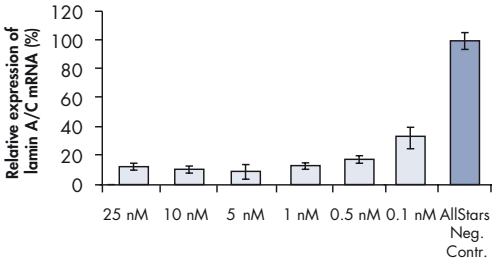

Figure 11. Rapid, efficient knockdown using preoptimized FlexiTube siRNA Premix. HepG2 cells were transfected with various amounts of FlexiTube siRNA Premix targeted against lamin A/C. After 48 hours, knockdown was analyzed by quantitative, real-time RT-PCR. All amounts resulted in highly efficient lamin A/C knockdown with no need for optimization.

Biology-on-Array Systems

For discovery of mechanisms controlling changes in gene expression

- Discover regulatory mechanisms in a single experiment
- Straightforward siRNA and real-time RT-PCR procedure
- Easily screen regulatory proteins involved in gene expression

The Biology-on-Array system identifies the proteins that regulate the expression of a gene. The system combines ready-to-transfect FlexiPlate siRNA with quantitative, real-time RT-PCR to yield a reverse genetics approach. Each Biology-on-Array siRNA plate disrupts the function of 84 relevant regulatory genes in parallel, then real-time PCR detects changes in gene expression associated with the knockdown of each regulatory protein. The Biology-on-Array analysis software simplifies analysis and identifies the mechanisms controlling the expression of the gene of interest. Visit www.SABiosciences.com (a QIAGEN company) for more information about Biology-on-Array.

Figure 12. Biology-on-Array analysis identifies TP53 as a main mediator of 5-fluorouracil mode of action. CDKN1A-specific real-time quantitative RT-PCR on 88 samples (84 samples after knockdown of 84 cancer-related transcription factors and 4 negative controls) showed that, under the influence of 5-fluorouracil (a cancer-treatment drug), knockdown of TP53 significantly down-regulated CDKN1A expression indicating that TP53 is a positive transcriptional regulator of CDKN1A. VDR and SMAD4 are also shown to be involved in 5-fluorouracil mediated up-regulation of CDKN1A.

SureSilencing shRNA Plasmids

For genomewide, plasmid-based RNAi

- Antibiotic selection allows stable plasmid transfection
- Experimentally verified algorithm ensures specificity and efficacy
- Complete shRNA sequence information provided
- Plasmids provide renewable source of RNA interference

SureSilencing shRNA Plasmids are available for any human, mouse, or rat gene, enabling specific, efficient gene knockdown using short-hairpin RNA. Five plasmids are provided for each gene: 4 gene-specific shRNA plasmids and one negative control plasmid. The choice of markers includes a GFP reporter gene, or neomycin, puromycin, or hygromycin markers. For projects where stable plasmid transfection is preferred and for the study of long-term knockdown effects, SureSilencing shRNA Plasmids provide an effective alternative to siRNA. Visit www.SABiosciences.com (a QIAGEN company) for more information about SureSilencing shRNA Plasmids.

Kits and assays for real-time RT-PCR

Primer sets and master mixes for SYBR® Green-based real-time RT-PCR

- Genomewide primer sets matched and standardized with siRNA
- PCR reagents for fast cycling with time savings of up to 60%
- High sensitivity and specificity ensure accurate quantification

QuantiTect Primer Assays are premixed, genomewide primer sets that provide highly specific and sensitive results in SYBR Green-based real-time RT-PCR. They have been tested in combination with QIAGEN siRNA for optimal performance and functionality. Both siRNAs and QuantiTect Primer Assays are available at GeneGlobe (www.qiagen.com/GeneGlobe). QuantiTect Primer Assays are available in tubes or in 96-well or 384-well plates. QuantiTect Primer Assays are used in combination with Rotor-Gene, QuantiFast, or QuantiTect SYBR Green Kits to carry out one-step or two-step quantitative, real-time RT-PCR with SYBR Green detection (Figure 13). QuantiFast SYBR Green Kits include an optimized master mix that delivers fast and specific quantification in real-time RT-PCR. Significantly reduced PCR run times are achieved not only on fast cyclers with rapid ramping rates, but also on all standard cyclers (www.qiagen.com/FastPCR). For ultrafast and precise real-time RT-PCR, Rotor-Gene SYBR Green Kits have been optimized for use on the Rotor-Gene Q cycler.

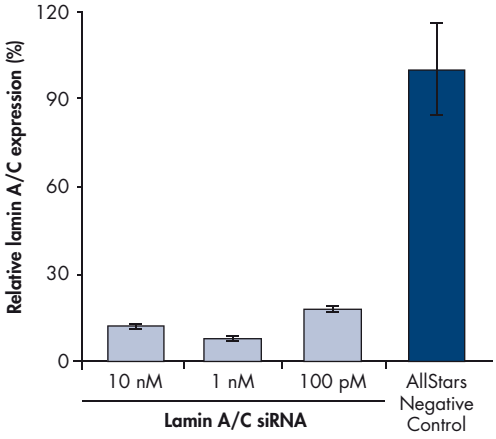

Figure 13. Validation of gene knockdown using the QuantiFast SYBR Green RT-PCR Kit. Normal Human Lung Fibroblasts were transfected with various amounts of siRNA targeted against lamin A/C using HiPerFect Transfection Reagent. AllStars Negative Control siRNA was also transfected. After 48 hours, knockdown was analyzed by quantitative, real-time one-step RT-PCR using the QuantiFast SYBR Green RT-PCR Kit in combination with the Lamin A/C QuantiTect Primer Assay.

Qproteome Kits

Easy-to-use kits for analyzing the impact of your gene silencing experiment at the protein level

- Confirm efficient knockdown at the protein level
- Detect changes in protein localization
- Detect changes in protein modifications
- Simple procedures with no specialized equipment required

A wide range of Qproteome Kits are available for whole proteome isolation from a variety of sample types, isolation of subproteomes based on cellular location, or isolation of modified proteins. See the complete range of sample prep kits at www.qiagen.com/Protein.

Ordering Information

Product	Contents	Cat. no.
FlexiTube siRNA	20 nmol, 5 nmol, or 1 nmol siRNA; delivered in tubes	Varies*
FlexiTube GeneSolution	4 siRNAs (1 nmol) recommended for a gene; delivered in tubes	Varies*
FlexiTube siRNA Premix	Optimized siRNA–reagent mix; 0.75 nmol siRNA	Varies*
FlexiPlate siRNA	siRNA in 96-well and 384-well plates; minimum order 36 siRNAs	Varies*
HP Custom siRNA	siRNA purified to >90% (20 nmol), modifications and labels available	Varies
Large-Scale siRNA Synthesis	siRNA from 10 mg to 10 g with labeling and modification options	Varies
QIAGEN AllStars RNAi Controls	Positive, negative, transfection, downstream, reporter, and interferon controls	Varies*
RNAi Human/Mouse Starter Kit	For >160 transfections in 24-well plates: HiPerFect Reagent, Positive and Negative Control siRNA	301799
HiPerFect Transfection Reagent (0.5 ml)†	Reagent for up to 166 transfections in 24-well plates	301704
QuantiTect Primer Assay (200)	Forward and reverse primer mix for SYBR Green-based real-time PCR	Varies*
QuantiFast SYBR Green PCR Kit (400)‡	Fast real-time PCR kit for 400 x 25 µl reactions	204054
Biology-on-Array Systems	siRNA plates, kit for first-strand synthesis, data analysis software	Varies¶
SureSilencing shRNA Plasmids	Gene-specific shRNA cloned into a plasmid containing a choice of markers	Varies¶

* Visit www.qiagen.com/GeneGlobe to search for and order these products. † Larger or smaller amounts and bulk quantities available; please inquire.
‡ Larger kit size and RT-PCR Kits available; please inquire. ¶ Visit www.SABiosciences.com (a QIAGEN company) to order these products.

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