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Getting started in RNAi research

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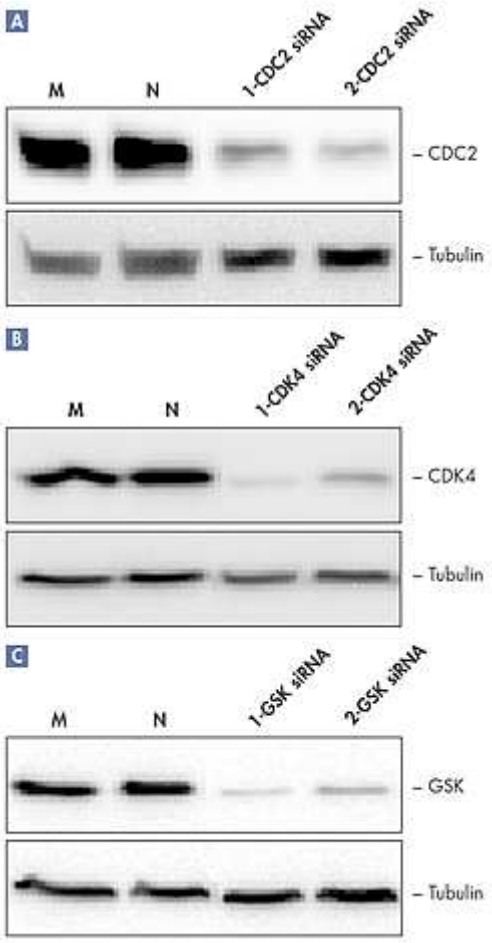
This article addresses the most important practical considerations for researchers who want to set up gene knockdown experiments, and offers tips, guidelines, and hints for a successful start in RNAi.

1. **Choose your cell line for transfection**
   First you should choose your cell line of interest and determine the appropriate culture format for your experiments. RNAiFect Transfection Reagent has been used successfully for siRNA transfection of a large number of cell lines and primary cell types. As part of our online transfection resources you can find a regularly updated list of successfully transfected cell types together with appropriate culture and transfection conditions. For siRNA transfection of suspension cells we recommend Amaxa Nucleofector technology.

2. **Choosing a synthetic siRNA**
   The major advantages of using chemically synthesized siRNA for gene knockdown are speed and convenience. QIAGEN provides library siRNA for common target genes that has been shown to deliver effective knockdown. siRNA duplexes can also be custom designed and synthesized with a short turnaround time. Chemically synthesized siRNA duplexes are subject to stringent quality control to ensure high purity. Another advantage of using synthetic siRNA is the possibility of knockdown experiments in cell lines that are difficult to transfect. siRNA is delivered directly to the cytoplasm and does not need to be directed to the nucleus, as in the plasmid-based approach. Labeling siRNA with fluorescent dyes allows easy monitoring of the uptake of the siRNA into the cell.

3. **The importance of good design**
   To achieve maximum levels of knockdown and minimize nonspecific effects, siRNA must be designed using a set of rules that take into account factors such as GC content, sequence, location, and homology to other genes. The algorithm used to design 2-for-Silencing siRNA Duplexes (discontinued; view updated siRNA design products) and Human Library siRNA Sets is highly sophisticated, and was developed using data obtained from a project that screened 3300 siRNA duplexes targeting 33 human genes. Highly potent siRNA allows efficient, specific knockdown. Optimally designed siRNA allows lower siRNA concentrations to be used, minimizing the risk of off-target effects. It also allows the gene silencing effect to be observed for longer time periods. The potency of siRNA designed using this algorithm enables QIAGEN to offer the Golden Guarantee of 100% satisfaction. By ordering siRNA that guarantees knockdown, the number of sequences that need to be tested decreases, significantly lowering costs. Western blot analysis shows the gene silencing effect of siRNA duplexes targeted against three genes and the high level of knockdown typically achieved using siRNA duplexes designed with the HiPerformance algorithm (see Figure "Efficient Knockdown Using siRNA Designed with the HiPerformance Algorithm") For researchers who want to design siRNA on their own, we also offer a free, easy-to-use web-based design algorithm.

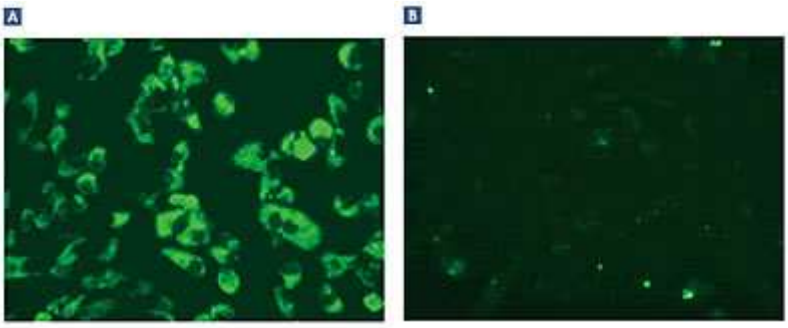
   Efficient knockdown Using siRNA Designed with the HiPerformance Algorithm



HeLa S3 cells were transfected with siRNA targeted against each of three genes: **A** *CDC2,* **B** *CDK4, and* **C** *GSK using RNAiFect Transfection Reagent. Cells were also mock transfected without siRNA addition (**M**) and transfected with a non-silencing control siRNA (**N**). After 48-72 hours cell lysates were separated by SDS-PAGE and analyzed by western blot using CDC2-, CDK4-, or GSK-specific antibodies. Blots were also probed with tubulin-specific antibody, as an internal control.*

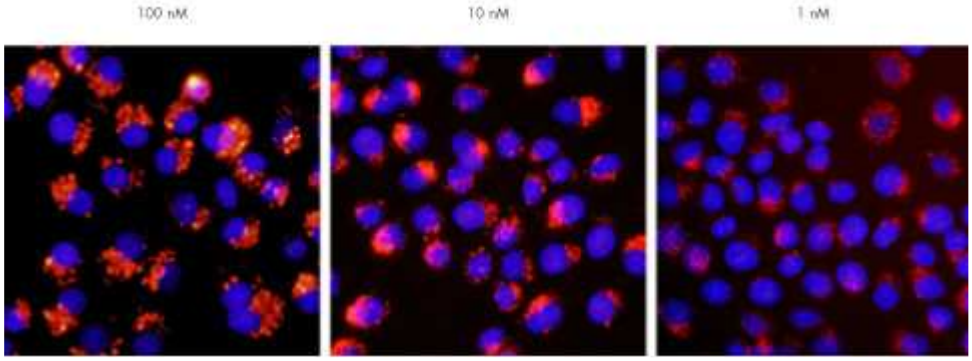
4. **Identify the optimal ratio of siRNA to transfection reagent and the optimal confluency of your cells**
The RNAi Human/Mouse Control Kit (discontinued; view updated product) allows convenient setup of RNAi experiments with appropriate positive and negative controls. Starting points for optimization trials using siRNA duplexes included in this kit are given in the *RNAi Human/Mouse Control Kit Handbook*. The non-silencing control siRNA is labeled with Alexa Fluor 488 allowing transfection efficiency to be monitored by fluorescence microscopy. Alexa Fluor fluorescent dyes have a longer duration of fluorescence than traditionally used dyes, and fluorescence is visible when Alexa Fluor labeled siRNA duplexes are transfected at low concentrations (see Figures "Alexa Fluor Label Allows Easy Monitoring of Transfection" and "Alexa Fluor Label is Visible in Transfected Cells at Low Concentrations"). Control Library siRNA Duplexes labeled with a range of Alexa Fluor dyes are available for monitoring transfection efficiency.
By varying the amount of RNAiFect Transfection Reagent in the presence of a fixed concentration of siRNA, the ratio of reagent to siRNA can be optimized. The recommended confluency for transfection of adherent cells with siRNA is 50-80%. However the optimal confluency should be determined for every new cell line to be transfected. Cells should be seeded 24 hours before transfection to ensure that the cell density is not too high and that the cells are in optimal physiological condition on the day of transfection.

Alexa Fluor Label Allows Easy Monitoring of Transfection



HeLa S3 cells 24 hours after transfection with **A** *Alexa Fluor 488 labeled siRNA and* **B** *FITC-labeled siRNA.*

Alexa Fluor Label is Visible in Transfected Cells at Low Concentrations



Fluorescence microscopy of HeLa S3 cells 48 hours after transfection with 100nM, 10 nM, or 1 nM non-silencing siRNA labeled at the 3' end of the sense strand with Alexa Fluor 546.

5. **Establish an assay for the detection and quantification of gene silencing**
   As a positive control for transfection experiments the RNAi Human/Mouse Control Kit contains an siRNA duplex targeting MAPK1. After transfection and incubation the RNeasy system can be used for purification of total RNA from a wide range of cell types. For downstream analysis QIAGEN offers validated gene expression assays for quantitative RT-PCR of human and mouse MAPK1. The Tag·100 Antibody can be used to detect both human and mouse MAPK1 in western blot procedures. After establishing the assay for your gene of interest, a final optimization experiment should be carried out with the specific siRNA under study. A range of siRNA concentrations should be used at optimized transfection conditions to determine the minimum siRNA amount necessary for efficient gene silencing. This amount can then be used in future experiments.

QIAGEN offers HiPerformance RNAi products and services for all stages of RNAi experiments, from siRNA design and delivery to downstream analysis. If you have any technical questions about RNAi or any QIAGEN products, contact your local Technical Services department.