RNA interference in mammalian cells using low siRNA concentrations

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Introduction

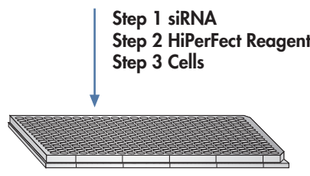
The use of short interfering RNA (siRNA) for knockdown of gene expression has become a powerful tool in molecular and cell biology. Some applications require the use of low siRNA concentrations (less than 5 nM), for example, to decrease the possibility of non-specific effects.

We have developed a transfection reagent, HiPerFect Transfection Reagent, which allows efficient gene knockdown with siRNA concentrations from 1 nM–10 nM, depending on the cell type and siRNA used. HiPerFect Transfection Reagent has been tested and validated for many cell types, including primary cells. Effective knockdown in primary cells demonstrates that HiPerFect Transfection Reagent ensures low cytotoxicity levels.

A Fast-Forward siRNA Transfection Protocol has been developed for rapid transfection with HiPerFect Transfection Reagent. This protocol allows cell seeding and transfection on the same day.

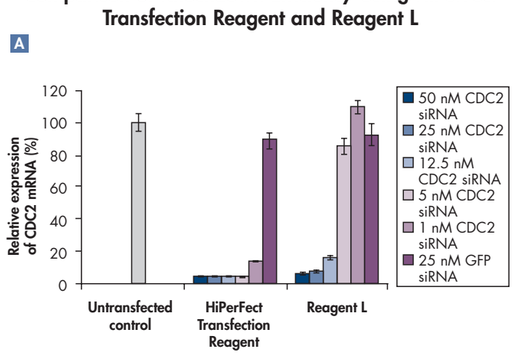
A reverse transfection protocol has been developed that is ideal for use in high-throughput applications. In reverse transfection, siRNA is spotted into wells, followed by addition of HiPerFect Reagent. After complex formation, cells are added to the wells.

Reverse Transfection Using HiPerFect Transfection Reagent

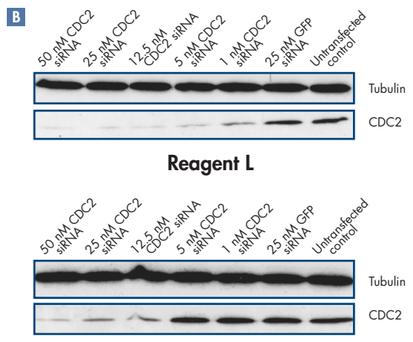


Highly effective knockdown of CDC2 expression with low siRNA concentrations

Comparison of Knockdown Efficiency Using HiPerFect Transfection Reagent and Reagent L



HiPerFect Transfection Reagent



Reagent L

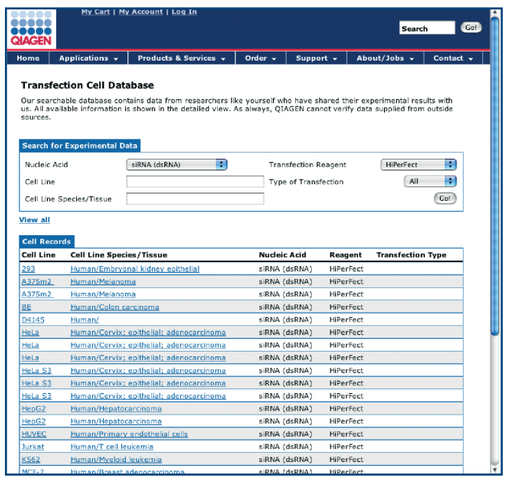
Figure 1 HeLa S3 cells were transfected with a range of concentrations of siRNA targeted against CDC2 using HiPerFect Transfection Reagent from QIAGEN or Reagent L from another supplier. Non-silencing siRNA targeting green fluorescent protein (GFP) was also transfected. After 48 hours, gene silencing was assessed by A quantitative, real-time RT-PCR and B western blot analysis. For RT-PCR analysis, total cellular RNA was purified using the RNeasy® system, and reverse transcribed using Omniscript® Reverse Transcriptase. The resultant cDNA was used for real-time RT-PCR. Expression of CDC2 was normalized to the expression of GAPDH. Values derived from real-time RT-PCR of control, untransfected cells were set as 100% and the relative expression levels of cells transfected with the experimental siRNA are indicated on the graph. For western blot analysis, cell lysates were separated by SDS-PAGE and analyzed by western blotting using CDC2-specific antibodies. Blots were also probed with tubulin-specific antibody as an internal control.

- HiPerFect Transfection Reagent from QIAGEN allowed highly efficient CDC2 knockdown with siRNA concentrations as low as 1 nM.
- In contrast, Reagent L from another supplier provided less efficient knockdown at all concentrations tested. For concentrations lower then 5 nM, knockdown of only 15% or less was observed.

Transfection and knockdown in a wide range of cell types

A wide range of cell types have been successfully transfected using HiPerFect Transfection Reagent. For an up-to-date list of cell types and more detailed information go to www.qiagen.com/TransfectionTools .

A Wide Range of Successfully Transfected Cells



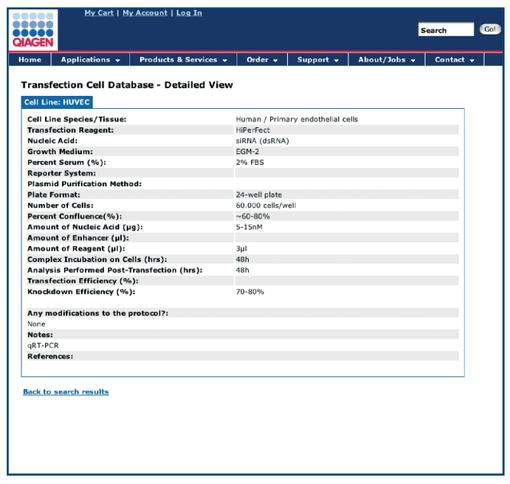


Figure 3 The complete cell list is available at www.qiagen.com/TransfectionTools .

Rapid, efficient lamin A/C knockdown in human primary cells

Lamin A/C Knockdown Using the Fast-Forward Protocol with Low siRNA Concentrations

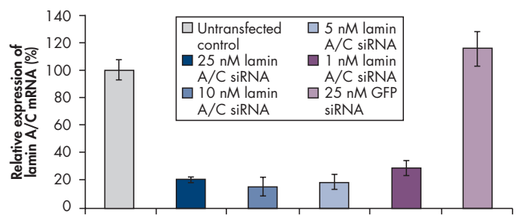


Figure 3 Normal Human Lung Fibroblasts (NHLF) were transfected with siRNA targeted against lamin A/C using HiPerFect Transfection Reagent with the Fast-Forward Protocol. The knockdown efficiency was analyzed by quantitative, duplex, one-step RT-PCR. Expression of lamin A/C was normalized to the expression of GAPDH and calculated relative to expression after transfection of non-silencing siRNA.

Microscopic Analysis of Transfected Cells

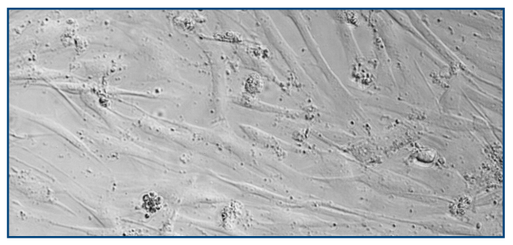
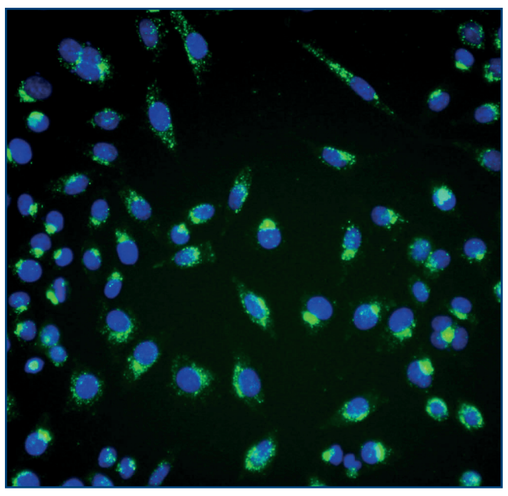


Figure 4 Phase contrast microscopy of transfected cells.

- HiPerFect Transfection Reagent allowed highly efficient lamin A/C knockdown with siRNA concentrations as low as 1 nM.
- The Fast-Forward Transfection Protocol allowed rapid transfections, with cell seeding and transfection carried out on the same day.
- Low cytotoxicity means that HiPerFect Transfection Reagent is especially suitable for use with sensitive primary cells.

HiPerFect Transfection Reagent Allows Effective Uptake of Low Amounts of Alexa Fluor® 488 Labeled siRNA

Fluorescently Labeled siRNA Shows Highly Efficient Uptake at Low siRNA Concentrations



- Microscopic analysis of fluorescently labeled siRNA showed siRNA was taken up into virtually all cells when HiPerFect Transfection Reagent was used.

Figure 5 Fluorescence microscopy of HeLa cells 24 hours after transfection with 5 nM Alexa Fluor 488 labeled siRNA (green fluorescence) using HiPerFect Transfection Reagent. Nuclei were stained with Hoechst 33342 (blue).

Summary

- **HiPerFect Transfection Reagent allows gene silencing using as little as 1 nM siRNA.**
  Transfection of low siRNA concentrations may be necessary to avoid off-target effects. Using HiPerFect Transfection Reagent means that effective knockdown can be achieved with very low siRNA concentrations.
- **HiPerFect Transfection Reagent has been tested for a range of cell types.**
  Many cell lines and primary cells have been successfully transfected using low siRNA concentrations and HiPerFect Transfection Reagent. For an up-to-date list, visit www.qiagen.com/TransfectionTools .
- **The Fast-Forward Protocol has been developed for rapid transfection.**
  In the Fast-Forward Protocol, cell seeding and siRNA transfection are carried out on the same day, saving time and effort. The Fast-Forward Protocol is available at www.qiagen.com/goto/HiPerFect .
- **The reverse transfection protocol is ideal for RNAi screening**
  The reverse transfection protocol can be easily automated which is particularly useful for high-throughput applications. The reverse transfection protocol is available at www.qiagen.com/goto/HiPerFect .

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