Seven Steps Toward RNAi Success

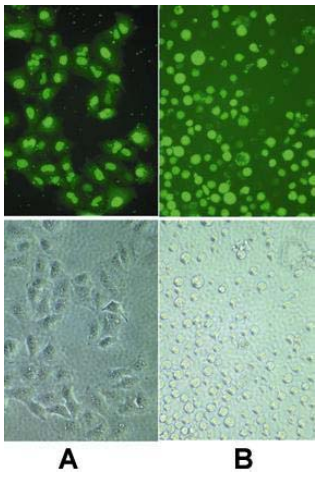
1. Optimize transfection conditions before beginning experiments with Stealth™ RNA.

The level of confluence and passage number of the cells at the start of transfection can have a significant impact on the efficiency of Stealth™ RNA uptake and on the cellular toxicity associated with transfection. Before beginning RNAi analysis, we recommend optimizing transfection conditions by determining the optimal cell density and oligomer-lipid concentrations to use for your mammalian cell line and system. When optimizing transfection conditions, follow the guidelines below:

- To ensure uniform uptake of Stealth™ RNA, make sure that cells are plated uniformly across the wells.
- For highly efficient transfection in a broad range of mammalian cell types, use Lipofectamine™ 2000 Reagent (Catalog no. 11668-027) available from Invitrogen (1).
- Use the BLOCK-iT™ Fluorescent Oligo (Catalog no. 2013) available from Invitrogen to optimize transfection conditions. The BLOCK-iT™ Fluorescent Oligo is a FITC-labeled, chemically-modified, dsRNA oligomer designed for use as an indicator of transfection efficiency. Uptake of the BLOCK-iT™ Fluorescent Oligo correlates strongly with uptake of Stealth™ RNA or siRNA oligomers. For more information, see www.invitrogen.com or call Technical Service.

2. Include the BLOCK-iT™ Fluorescent Oligo in every experiment.

The degree of the RNAi response to a particular Stealth™ RNA or siRNA oligomer is directly linked to its transfection efficiency. To assess transfection efficiency, we recommend including the BLOCK-iT™ Fluorescent Oligo in every experiment. Using the BLOCK-iT™ Fluorescent Oligo in your transfection experiment allows you to easily assess oligomer uptake and transfection efficiency using any fluorescence microscope and a standard FITC filter set. Uptake of the fluorescent oligomer by at least 80% of cells correlates with high levels of gene knockdown by effective Stealth™ RNA or siRNA oligomers. For examples of good fluorescent uptake in adherent (panel A) and suspension (panel B) cells, see the figure on the right.



Note: The BLOCK-iT™ Fluorescent Oligo is chemically modified to enhance its stability and allows assessment of fluorescence signal for a significantly longer time period than is obtained with other unmodified, fluorescently-labeled RNA.

3. Assess Stealth™ RNAi or siRNA effects by performing an RNA assay (*i.e.* qRT-PCR) first.

To validate your Stealth™ RNA or siRNA oligomers, you must measure each oligomer's effect on the target mRNA. Many investigators wish to bypass the RNA determination step and look directly at the Stealth™ RNA or siRNA oligomer's effect on protein levels. We strongly advise against this, as the RNA assay will yield important information about the rank order potency of the oligomers against the target mRNA and provides valuable information required to troubleshoot the assay system. For example, an RNAi oligomer may be effective at decreasing mRNA levels of the target gene; however, may not affect protein levels if the target protein has a long half-life.

Quantitative RT-PCR (qRT-PCR) using Invitrogen's custom LUX™ primers provides a convenient and high throughput method to evaluate the effect of an individual or set of Stealth™ RNA or siRNA oligomers on target mRNA levels. Use the LUX™ Designer available at www.invitrogen.com/lux to help you design and order suitable primers for use in qRT-PCR analysis. To prepare mRNA or total RNA from untreated or oligomer-treated cells, use Invitrogen's mRNA Catcher™ Kit (Catalog no. 7001) or Concert™ 96 RNA Purification System (Catalog no. 12173-011), respectively. When performing qRT-PCR, remember to normalize results to an internal control RNA (*e.g.* β-actin or GAPDH).

4. Know the half-life of the protein that you wish to inhibit.

To see Stealth™ RNA or siRNA-mediated inhibition at the protein level, any pre-existing pool of the protein must be degraded. If the protein of interest has a long half-life, you may need to perform long-term transfection experiments (*i.e.* perform multiple cycles of transfection) to observe effects at the protein level.

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5. Always include the appropriate positive and negative controls.

When performing RNAi analysis, it is important to include the proper positive and negative controls to help you evaluate your results. For a positive control, include an effective Stealth™ RNA for a target other than your mRNA of interest. For a negative control, compare the levels of the target mRNA in Stealth™ RNA or siRNA-treated and control (scrambled or reverse sequences)-treated cells. If you are performing RNAi analysis in human cells, Invitrogen offers the BLOCK-iT™ Transfection Optimization Kit (Catalog no. 13750-047) to help you optimize RNAi transfection using controls for transfection and viability. The kit includes a Stealth™ RNA molecule targeting the human p53 gene for use as a positive control and a Scrambled Stealth™ RNA molecule for use as a negative control.

6. Follow these general guidelines to perform RNAi analysis using Stealth™ RNA or siRNA.

- When preparing oligomer-lipid complexes, dilute oligomer and lipid into the appropriate medium. We recommend using Opti-MEM® I Reduced Serum Medium (Catalog no. 31985-070) available from Invitrogen. **Do not** use phosphate-buffered saline (PBS) for dilution, as transfection efficiency will be severely compromised.
- Always mix the Stealth™ RNA or siRNA oligomer stock solution thoroughly before use. Thaw, vortex, and spin to collect fluid before removing sample.
- Do not allow the cells to dry out before adding oligomer-lipid complexes. Doing so will reduce the transfection efficiency and cell viability.

For detailed protocols to transfect Stealth™ RNA or siRNA oligomers, refer to the manufacturer's instructions for the transfection reagent you are using.

7. Visit our Web site at www.invitrogen.com/rnai for additional information, resources, and protocols to help you achieve success in your RNAi analysis.

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References

1. Ciccarone, V., Chu, Y., Schifferli, K., Pichet, J.-P., Hawley-Nelson, P., Evans, K., Roy, L., and Bennett, S. (1999) *Focus* 21, 54-55.