

Stealth RNAi® Negative Control Duplexes

 Package Contents	Size 20 µM stock in solution  Available Duplexes <ul style="list-style-type: none"> 1.75 mL Nuclease-free Water 1× RNA Annealing/Dilution Buffer
 Storage Conditions	<ul style="list-style-type: none"> Store at or below –20°C. Do not store in a frost-free freezer. (Dried oligonucleotides are shipped at room temperature.)
 Required Materials	<ul style="list-style-type: none"> RNase-free reagents Transfection reagent e.g. Lipofectamine® RNAiMAX
 Timing	Transfection preparation: 15 minutes Final incubation: 1–3 days
 Selection Guide	siRNAs Go online to view related products.
 Product Description	<ul style="list-style-type: none"> Stealth RNAi® are chemically modified, 25-mer, double-stranded RNA, with no overhangs, that show effective knockdown and have a low off target profile. Stealth RNAi® Negative Control Duplexes are ideal for use in RNA interference (RNAi) experiments as a control for sequence independent effects following Stealth RNAi® delivery in any vertebrate cell line.
 Important Guidelines	<ul style="list-style-type: none"> Handling instructions: RNA oligonucleotides are susceptible to degradation by exogenous ribonucleases introduced during handling. Wear gloves when handling this product. Use RNase-free reagents, tubes, and barrier pipette tips. Transfection efficiency varies according to the cell type and transfection agent used. We recommend starting with a 10 nM final concentration of the Stealth RNAi® Negative Control Duplex to determine the optimal conditions for your desired outcomes. Transfect Stealth RNAi® Negative Control Duplex using the same methodology as for your experimental siRNA duplexes.
 Online Resources	Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support . 

For Research Use Only. Not for use in diagnostic procedures.

Stealth RNAi® Negative Control Duplex Characteristics

This control is designed for use in RNAi analysis to facilitate assessment and optimization of cationic lipid-mediated delivery. It has the following characteristics:

- Our Stealth RNAi® Duplexes have a proven correlation of transfection efficiency with siRNA molecules.
- Each Stealth RNAi® Negative Control Duplex is designed to minimize sequence homology to any known vertebrate transcript.
- The Stealth RNAi® Negative Control Duplexes differ from one another in their GC content, and are supplied in a ready-to-use format.
- A 1× RNA Annealing/Dilution Buffer is included for dilution of the Stealth RNAi® stock solution, if desired.

RNAi Transfection Protocol

- See page 2 to view guidelines for transfecting siRNAs using Lipofectamine® RNAiMAX Reagent.

Transfection Amounts per Well

Use 10 nM siRNA duplex as a starting point.

	96-well	24-well	6-well
Final siRNA	1 pmol	5 pmol	25 pmol
Final Lipofectamine® RNAiMAX	0.3 µL	1.5 µL	7.5 µL

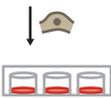
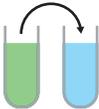
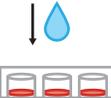
Limited Product Warranty and Disclaimer Details

Limited Use Label License

RNAi Transfection Protocol

This procedure is designed for one RNA amount combined with one amount of Lipofectamine® RNAiMAX.

The prepared mix is enough to have triplicates (96-well), duplicates (24-well), and single well (6-well) transfections, and account for pipetting variations.

Timeline			Steps	Procedure Details			
Day 0	1		Seed cells to be 60-80% confluent at transfection	Component	96-well	24-well	6-well
	2		Dilute Lipofectamine® RNAiMAX Reagent in Opti-MEM® Medium	Adherent cells	1–4 × 10 ⁴	0.5–2 × 10 ⁵	0.25–1 × 10 ⁶
Day 1	3		Dilute siRNA in Opti-MEM® Medium	Opti-MEM® Medium	25 µL	50 µL	150 µL
	4		Add diluted siRNA to diluted Lipofectamine® RNAiMAX Reagent (1:1 ratio)	Lipofectamine® RNAiMAX Reagent	1.5 µL	3 µL	9 µL
	5		Incubate	Opti-MEM® Medium	25 µL	50 µL	150 µL
Day 2–4	6		Add siRNA-lipid complex to cells	siRNA (10 µM)	0.5 µL (5 pmol)	1 µL (10 pmol)	3 µL (30 pmol)
	7		Visualize/analyze transfected cells	Diluted siRNA	25 µL	50 µL	150 µL
				Diluted Lipofectamine® RNAiMAX Reagent	25 µL	50 µL	150 µL
				Incubate for 5 minutes at room temperature.			
				Component	96-well	24-well	6-well
				siRNA-lipid complex per well	10 µL	50 µL	250 µL
				Final siRNA used per well	1 pmol	5 pmol	25 pmol
				Final Lipofectamine® RNAiMAX used per well	0.3 µL	1.5 µL	7.5 µL
				Incubate cells for 1–3 days at 37°C. Then, analyze transfected cells.			