

BLOCK-iT™ Fluorescent Control

	Package Contents	Catalog Number	Size
		2013 <i>Lipid Transfection</i>	20 µM stock; 2 × 125 µL
		13750-062 <i>Electroporation</i>	1 mM stock; 75 µL
		▪ 1.75 mL Nuclease-free Water	
	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® 2000 	
	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"> ▪ BLOCK-iT™ Fluorescent Control is a fluorescein-labeled (green), dsRNA oligomer designed for use in RNAi analysis to facilitate assessment and optimize delivery of dsRNA oligonucleotides into mammalian cells. ▪ We do not recommend using Lipofectamine® RNAiMAX reagent with the BLOCK-iT™ Fluorescent Control. For Lipofectamine® RNAiMAX reagent, we recommend using the BLOCK-iT™ Alexa Fluor® Red Fluorescent Control Cat. No. 14750-100. 	
	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. If you are transfecting your cell line for the first time, we recommend starting with a 10 nM final concentration of the BLOCK-iT™ Fluorescent Control to determine the optimal amount for a strong fluorescence signal. ▪ Transfect BLOCK-iT™ Fluorescent Control using the same methodology as for your experimental siRNA duplexes. 	

BLOCK-iT™ Fluorescent Control Characteristics

This control is designed for use in RNAi analysis to facilitate assessment and optimization of cationic lipid-mediated delivery or electroporation of dsRNA oligonucleotides into mammalian cells. It has the following characteristics:

- Chemical modifications that enhance the stability and allow assessment of fluorescence signal for a significantly longer time than is obtained with other unmodified, fluorescently labeled RNA. It has the same length, charge, and configuration as standard siRNA.
- Proven correlation of transfection efficiency with siRNA molecules.
- Localization primarily to the nucleus upon uptake, designed strictly for use as a tool for siRNA uptake assessment.
- No significant sequence similarity to mouse, rat, or human transcript sequences and has been tested in multiple cell lines and shown to have no significant impact on cell proliferation, apoptosis, or cell morphology.

RNAi Transfection Protocol

- ❗ See page 2 to view guidelines for transfecting siRNAs using Lipofectamine® 2000 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® 2000 Reagent	0.3 µL	1.5 µL	7.5 µL

❗ Limited Product Warranty and Disclaimer Details.

 **Online Resources** Visit our [product page](#) for additional information and protocols. For support, visit www.lifetechnologies.com/support.

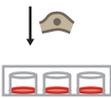
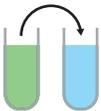
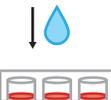


For Research Use Only. Not for use in diagnostic procedures.

RNAi Transfection Protocol

This procedure is designed for one RNA amount combined with one amount of Lipofectamine® 2000 Reagent.

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® 2000 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® 2000 Reagent (1:1 ratio)	Lipofectamine® 2000 Reagent	1.5 µL	3 µL	9 µL
	5		Incubate	Opti-MEM® Medium	25 µL	50 µL	150 µL
	6		Add siRNA-lipid complex to cells	siRNA (10 µM)	0.5 µL (5 pmol)	1 µL (10 pmol)	3 µL (30 pmol)
Day 2-4	7		Visualize/analyze transfected cells	Diluted siRNA	25 µL	50 µL	150 µL
				Diluted Lipofectamine® 2000 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® 2000 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.			