# BLOCK-iT™ Alexa Fluor® Red Fluorescent Control

**Package Contents**

| Catalog Number | Size |
|---|---|
| 14750-100 | 20 µM stock in solution |

- 1.75 mL Nuclease-free Water

**Storage Conditions**

- Store at or below –20°C.
- Do not store in a frost-free freezer. (Dried oligonucleotides are shipped at room temperature.)

**Required Materials**

- 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**

- BLOCK-iT™ Alexa Fluor® Red Fluorescent Control is an Alexa Fluor® 555-labeled, double-stranded, RNA (dsRNA) duplex for assessing lipid-mediated transfection for RNAi experiments.
- Alexa Fluor® 555 dye can be detected using standard filter sets designed for Cy®3, DsRed, Texas Red®, or rhodamine fluorophores.

**Important Guidelines**

- 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™ Alexa Fluor® Red Fluorescent Control to determine the optimal amount for a strong fluorescence signal.
- Transfect BLOCK-iT™ Alexa Fluor® Red Fluorescent Control 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.

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Print Options

## BLOCK-iT™ Alexa Fluor® Red 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® 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

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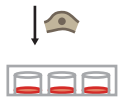
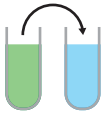
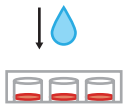
# RNAi Transfection Protocol

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 |
|---|---|---|
| Day 0 | 1  | **Seed cells to be 60-80% confluent at transfection** |
| | 2  | **Dilute Lipofectamine® RNAiMAX Reagent in Opti-MEM® Medium** |
| | 3  | **Dilute siRNA in Opti-MEM® Medium** |
| Day 1 | 4  | **Add diluted siRNA to diluted Lipofectamine® RNAiMAX Reagent (1:1 ratio)** |
| | 5  | **Incubate** |
| | 6  | **Add siRNA-lipid complex to cells** |
| Day 2–4 | 7  | **Visualize/analyze transfected cells** |

**Procedure Details**

| Component | 96-well | 24-well | 6-well |
|---|---|---|---|
| Adherent cells | 1–4 × $10^4$ | 0.5–2 × $10^5$ | 0.25–1 × $10^6$ |
| Opti-MEM® Medium | 25 µL | 50 µL | 150 µL |
| Lipofectamine® RNAiMAX Reagent | 1.5 µL | 3 µL | 9 µL |
| Opti-MEM® Medium | 25 µL | 50 µL | 150 µL |
| siRNA (10 µM) | 0.5 µL (5 pmol) | 1 µL (10 pmol) | 3 µL (30 pmol) |
| 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. | | | |