invitrogen™

BLOCK-iT™ Transfection Optimization Kit

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User Manual

Table of Contents

Kit Contents and Storage

Shipping and Storage

The BLOCK-iT™ Transfection Optimization Kit is shipped on blue ice. Upon receipt, store each reagent as detailed below.

Reagent	Storage
BLOCK-iT™ Fluorescent Oligo	-20°C, protected from light
Control Stealth™ RNAi Molecules	-20°C
Dead Cell Reagent	-20°C, protected from light

Contents

The BLOCK-iT™ Transfection Optimization Kit includes the following reagents. The annealing buffer is composed of 100 mM KOAc, 30 mM HEPES-KOH, pH 7.4, and 2 mM MgOAc. **Store the Stealth™ RNAi molecules at -20°C and the BLOCK-iT™ Fluorescent Oligo and the Dead Cell Reagent at -20°C, protected from light.**

Reagent	Composition	Amount
BLOCK-iT™ Fluorescent Oligo	20 µM FITC-labeled, double-stranded RNA oligomer in annealing buffer	2 x 125 µl
p53 Positive Control Stealth™ RNA	20 µM Stealth™ RNA in annealing buffer	125 µl
Scrambled Negative Control Stealth™ RNA	20 µM Stealth™ RNA in annealing buffer	125 µl
Dead Cell Reagent	2 mM Ethidium homodimer-1 (EthD-1) in DMSO/H_2O 1:4 (v/v)	200 µl

Accessory Products

Accessory Products

Some of the reagents supplied in the BLOCK-iT™ Transfection Optimization Kit as well as other products suitable for use with the kit are available separately from Invitrogen. Ordering information is provided below. For more information, refer to our Web site (www.invitrogen.com) or call Technical Service (see page 10).

Note: Some reagents are available in other sizes.

Item	Amount	Catalog no.
BLOCK-iT™ Fluorescent Oligo	2 x 125 µl (20 µM)	2013
	75 µl (1 mM)	13750-062
Ethidium Homodimer-1 (Dead Cell Reagent)	1 mg	E-1169
Lipofectamine™ 2000 Reagent	0.75 ml	11668-027
	1.5 ml	11668-019
Opti-MEM® I Reduced Serum Medium	100 ml	31985-062
	500 ml	31985-070

Introduction

Overview

Introduction

The BLOCK-iT™ Transfection Optimization Kit is designed to facilitate optimization of transfection conditions for RNAi studies. The kit provides the following reagents:

- BLOCK-iT™ Fluorescent Oligo, a FITC-labeled double-stranded RNA (dsRNA) oligomer for use as an indicator of transfection efficiency in RNAi experiments with Stealth™ RNA or siRNA
- A Stealth™ RNA molecule targeting the human p53 gene for use as a positive control (in human cell lines only) for the RNAi response
- A Scrambled Stealth™ RNA molecule for use as a negative control (in human cell lines only) for the RNAi response
- Dead Cell Reagent to assess cell viability

For more information about each reagent, see pages 2-3.

Uses for the BLOCK-iT™ Transfection Optimization Kit

Use the BLOCK-iT™ Transfection Optimization Kit in your RNAi studies for the following purposes:

- If you are transfecting Stealth™ RNA or siRNA into a mammalian cell line of interest for the first time, use the BLOCK-iT™ Fluorescent Oligo and the Dead Cell Reagent to help you optimize your transfection conditions.

  Note: If you are transfecting a human cell line, you may use the p53 and Scrambled Stealth™ RNA oligomers as positive and negative controls for the RNAi response. You will need to obtain the appropriate reagent(s) required to detect human p53 mRNA or protein (see page 9 for more information).

- Once you have determined the optimal conditions to use for transfection, include the BLOCK-iT™ Fluorescent Oligo and the Dead Cell Reagent in every RNAi experiment as an indicator of transfection efficiency and cell viability.

continued on next page

Overview, continued

BLOCK-iT™ Fluorescent Oligo

The BLOCK-iT™ Fluorescent Oligo allows strong, easy fluorescence-based assessment of dsRNA oligomer uptake into mammalian cells. The Oligo possesses the following characteristics:

- Is a FITC-labeled, double-stranded RNA duplex with the same length, charge, and configuration as standard siRNA.
- Contains chemical modifications that enhance the stability and allow assessment of fluorescence signal for a significantly longer time period than is obtained with other unmodified, fluorescently labeled RNA. **Example:** Fluorescence signal is readily detectable in HEK293 cells for at least 72 hours. Note that the strength of the fluorescence signal depends on the transfection efficiency, growth rate of the cells, and the amount of oligomer transfected.
- The sequence of the BLOCK-iT™ Fluorescent Oligo is not homologous to any known gene, ensuring against induction of non-specific cellular events caused by introduction of the Oligo into cells.
- Localizes primarily to the nucleus upon uptake (Fisher *et al.*, 1993).

Important

The BLOCK-iT™ Fluorescent Oligo is designed strictly for use as a tool for Stealth™ RNA or siRNA uptake assessment, and is not meant to provide any information about the behavior of your Stealth™ RNA or siRNA including its cellular localization, half-life, or stability.

continued on next page

Overview, continued

Control Stealth™ RNAi

The BLOCK-iT™ Transfection Optimization Kit includes a p53 and a Scrambled Stealth™ RNA molecule for use as positive and negative controls, respectively, in an RNAi experiment targeting the human p53 gene. If p53 is expressed in your human cell line of interest, we recommend using the two Stealth™ RNA molecules to help you optimize your transfection conditions. For more information about Stealth™ RNAi, see below.

Note: Do not use the p53 and Scrambled Stealth™ RNA molecules as controls in non-human cell lines.

Stealth™ RNAi

Stealth™ RNAi is chemically modified dsRNA developed to overcome the limitations of traditional siRNA. Using Stealth™ RNA for RNAi analysis offers the following advantages:

- Obtain effective target gene knockdown at levels that are equivalent to or greater than those achieved with traditional siRNA
- Reduces non-specific effects caused by induction of cellular stress response pathways
- Exhibit enhanced stability for greater flexibility in RNAi analysis

Dead Cell Reagent

Dead Cell Reagent is intended for use as an indicator of cell viability following transfection of mammalian cells with Stealth™ RNA or siRNA, and is an ethidium dye (ethidium homodimer-1; EthD-1) with the following characteristics:

- Molecular formula: $C_{46}H_{50}Cl_4N_8$
- Molecular weight: 856.77

Dead Cell Reagent enters cells with damaged membranes and emits a red fluorescence signal upon binding to nucleic acids (λ_{ex} = 528 nm, λ_{em} = 617 nm). The fluorescence signal is detectable using a fluorescence microscope and filters for propidium iodide or Texas Red®.

Note: Dead Cell Reagent is excluded by the intact plasma membrane of live cells.

Methods

Handling the Reagents

Introduction

Follow the guidelines below when handling the reagents supplied in the kit.

Handling the BLOCK-iT™ Fluorescent Oligo and Control Stealth™ RNAi

The BLOCK-iT™ Fluorescent Oligo and the control Stealth™ RNA molecules are each supplied as a 20 µM stock solution in an annealing buffer. Follow the guidelines below when handling the BLOCK-iT™ Fluorescent Oligo and Stealth™ RNA stock solutions.

- **Storage:** Store the BLOCK-iT™ Fluorescent Oligo stock solution at -20°C, protected from light, and the control Stealth™ RNA stock solutions at -20°C. All stock solutions are stable for at least 6 months if stored properly; Stealth™ RNAi duplex stock solutions are stable for 5 years if stored properly.
- **Thawing:** When using, thaw the stock solution on ice or at room temperature. Once thawed, place the tube on ice until use. After use, return stock solution to -20°C storage.
- **Freeze/thaw cycles:** The stock solution may be frozen and thawed multiple times without loss of fluorescence signal (BLOCK-iT™ Fluorescent Oligo) or activity (Control Stealth™ RNAi oligomers) if handled properly.
- **RNase-free conditions:** Take precautions to ensure that the stock solution does not become contaminated with RNase.
    1. Use RNase-free sterile pipette tips and supplies for all manipulations.
    2. Wear gloves when handling reagents and solutions.

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Handling the Reagents, continued

Handling the Dead Cell Reagent

The Dead Cell Reagent is supplied as a 2 mM stock solution in DMSO/H_2O 1:4 (v/v). Follow the guidelines below when handling the Dead Cell Reagent.

- The Dead Cell Reagent is light sensitive. Store the stock solution at -20°C, protected from light. The stock solution is stable for at least 6 months if stored properly.
- When using, thaw the stock solution at room temperature. Tap the tube to mix the stock solution, and centrifuge briefly before opening. After use, return stock solution to -20°C storage.
- The stock solution may be frozen and thawed multiple times without loss of fluorescence signal if handled properly.

Optimizing Transfection

Introduction

You may use any suitable cationic lipid-based transfection reagent to deliver Stealth™ RNA or siRNA to mammalian cells. This section provides general guidelines to use the reagents supplied in the kit to help you optimize transfection conditions for your Stealth™ RNA or siRNA and mammalian cell line.

For highly efficient delivery of Stealth™ RNA or siRNA to a wide variety of mammalian cells, we recommend using Lipofectamine™ 2000 Reagent (Gitlin *et al.*, 2002; Yu *et al.*, 2002) available from Invitrogen (see page vi for ordering information).

General Guidelines for Use

Follow the guidelines below when performing transfection:

- Determine the appropriate amount of each reagent to use such that fluorescence signal (BLOCK-iT™ Fluorescent Oligo or Dead Cell Reagent) or gene knockdown effect (p53 Stealth™ RNA) is readily detectable. For recommended reagent amounts to use, see the next page.
- When setting up your transfection experiment, we recommend transfecting one set of cells with the BLOCK-iT™ Fluorescent Oligo, then staining those cells with the Dead Cell Reagent at a suitable time period after transfection (generally 6 to 24 hours post-transfection). This allows simultaneous assessment of transfection efficiency and cell viability with the same sample.
- Prepare and seed cells at a density recommended by the manufacturer of the transfection reagent you are using.
- Prepare lipid-oligomer complexes as directed by the manufacturer of the transfection reagent you are using. Always dilute the BLOCK-iT™ Fluorescent Oligo or Stealth™ RNA immediately before transfection (*i.e.* do not store diluted oligomer) and into an appropriate medium. We recommend diluting the oligomer into Opti-MEM® I Reduced Serum Medium (see page vi for ordering information) available from Invitrogen.

continued on next page

Optimizing Transfection, continued

Amount of BLOCK-iT™ Fluorescent Oligo to Use

The amount of BLOCK-iT™ Fluorescent Oligo to transfect depends on the growth rate and transfection efficiency of the mammalian cells. To optimize transfection conditions, evaluate several concentrations of lipid and vary the final concentration of the BLOCK-iT™ Fluorescent Oligo from 10 to 200 nM to determine the optimal amount of Oligo required to obtain a strong fluorescence signal.

Note: As a starting point, we recommend using 100 nM BLOCK-iT™ Fluorescent Oligo.

Amount of Control Stealth™ RNA to Use

The amount of p53 Stealth™ RNA to transfect to achieve optimal gene knockdown needs to be determined experimentally for each human cell line. To optimize transfection conditions, evaluate several concentrations of lipid and vary the final concentration of Stealth™ RNA from 10 to 100 nM to determine the conditions required for the optimal levels of gene knockdown. Use of higher concentrations of Stealth™ RNA may be possible depending on the cell line.

Note: As a starting point, we recommend using 40 nM p53 Stealth™ RNA. Use the same concentration of the negative control Scrambled Stealth™ RNA.

continued on next page

Optimizing Transfection, continued

Staining Cells with Dead Cell Reagent

Follow this procedure to stain cells with Dead Cell Reagent. Prepare a sufficient amount of the working solution based on the number of samples you wish to stain (see table below).

1. Thaw the 2 mM Dead Cell Reagent stock solution at room temperature. Tap the tube to mix, and centrifuge briefly before opening.
2. Dilute the appropriate amount of Dead Cell Reagent into Opti-MEM® I Reduced Serum Medium to prepare a 2 µM working solution (1:1000 dilution).

   Example: To prepare 1 ml of a 2 µM working solution, add 1 µl of Dead Cell Reagent to 1 ml of Opti-MEM® I Reduced Serum Medium.
3. Aspirate the media from the cells and replace with the appropriate volume of Dead Cell Reagent (see table below).

Culture Vessel	Volume of Staining Solution per Well
6-well	2 ml
12-well	1 ml
24-well	0.5 ml
48-well	0.25 ml

4. Incubate cells at 37°C in a CO_2 incubator for 10-15 minutes.
5. Remove the Dead Cell Reagent and replace with fresh Opti-MEM® I Reduced Serum Medium.
6. Evaluate fluorescence signal using a fluorescence microscope and the appropriate filter set (see the next page).

Detecting p53 or Fluorescence Signal

Detecting p53 mRNA or Protein

You may use any method of choice to detect human p53 expression levels after treatment with the positive control Stealth™ RNA or the negative control scrambled Stealth™ RNA.

- To assay for p53 mRNA levels, we recommend performing quantitative RT-PCR (qRT-PCR) using Invitrogen's custom LUX™ primers. Use the LUX™ Designer available at www.invitrogen.com/lux to help you design and order suitable primers to use for qRT-PCR analysis. To prepare mRNA from treated or untreated cells, use Invitrogen's mRNA Catcher™ Kit (Catalog no. 7001). When performing qRT-PCR, remember to normalize results to an internal control RNA (*e.g.* β-actin, GAPDH, or cyclophilin).
- To assay for p53 protein levels, we recommend performing Western blot analysis using a suitable antibody to human p53. Remember to take into account the half-life of the protein when assessing RNAi effects at the protein level.

Detecting Fluorescence Signal

Once you have transfected your mammalian cells with the BLOCK-iT™ Fluorescent Oligo and have stained the cells with Dead Cell Reagent, you may qualitatively assess Oligo uptake and cell viability using any fluorescence microscope and the following filter sets:

- To assess transfection efficiency, use any standard FITC filter set (λ_{ex} = 494 nm, λ_{em} = 519 green) to detect the fluorescence signal from the BLOCK-iT™ Fluorescent Oligo
- To assess cell viability, use a filter set for propidium iodide or Texas Red® (λ_{ex} = 528 nm, λ_{em} = 617 nm) to detect the fluorescence signal from the Dead Cell Reagent

Appendix

Technical Service

World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

Contact Us

For more information or technical assistance, please call, write, fax, or email. Additional international offices are listed on our web page (www.invitrogen.com).

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continued on next page

Technical Service, continued

MSDS Requests

Material Safety Data Sheets (MSDSs) are available on our website at www.invitrogen.com/msds.

Certificate of Analysis

Product qualification is described in the Certificate of Analysis (CofA), available on our website by product lot number at www.invitrogen.com/cofa

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Introduction

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References

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