Invitrogen™
life technologies

Instruction Manual

BLOCK-iT™ Transfection Kit

Catalog no. 13750-070

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Kit Contents and Storage

Shipping/ Storage

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

- **BLOCK-iT™ Fluorescent Oligo:** -20°C, protected from light
- **Lipofectamine™ 2000:** +4°C (**do not freeze**)

Contents

The BLOCK-iT™ Transfection Kit includes the following reagents.

Reagent	Composition	Amount
BLOCK-iT™ Fluorescent Oligo	20 µM fluorescein-labeled, double-stranded RNA oligo in: 100 mM KOAc 30 mM HEPES-KOH, pH 7.4 2 mM MgOAc	125 µl
Lipofectamine™ 2000 Reagent	Proprietary	250 µl

Accessory Products

Accessory Products

The reagents supplied in the BLOCK-iT™ Transfection 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
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
Dead Cell Reagent (Ethidium Homodimer-1)	1 mg	E-1169

Overview

Introduction

The BLOCK-iT™ Transfection Kit is designed to facilitate optimization of cationic lipid-mediated delivery of Stealth™ RNA or standard siRNA to mammalian cells for RNAi analysis. The kit provides the following reagents:

- BLOCK-iT™ Fluorescent Oligo, a fluorescein-labeled double-stranded RNA (dsRNA) oligomer for use as an indicator of transfection efficiency in RNAi experiments with Stealth™ RNA or siRNA
- Lipofectamine™ 2000 Reagent for highly efficient delivery of dsRNA oligomers to a wide variety of mammalian cells

For more information about each reagent, see pages 2-3. For more information about Stealth™ RNA, see page 3.

Uses for the BLOCK-iT™ Transfection Kit

- Use Lipofectamine™ 2000 for highly efficient delivery of dsRNA oligomers including the BLOCK-iT™ Fluorescent Oligo and Stealth™ RNA or standard siRNA into your mammalian cell line of interest for RNAi analysis.
- Include the BLOCK-iT™ Fluorescent Oligo in your RNAi experiments to help you optimize your transfection conditions and to assess transfection efficiency in your mammalian cell line. Once you have optimized your transfection conditions, include the BLOCK-iT™ Fluorescent Oligo in every RNAi experiment as an indicator of transfection efficiency.

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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 fluorescein-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).
- Fluorescence signal is detectable using any fluorescence microscope and a standard FITC filter set.

The BLOCK-iT™ Fluorescent Oligo is supplied with the BLOCK-iT™ Transfection Kit, but is also available separately from Invitrogen (see page vi for ordering information).

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.

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Overview, continued

Lipofectamine™ 2000 Reagent

Lipofectamine™ 2000 Reagent is a proprietary, cationic lipid-based formulation suitable for delivery of the BLOCK-iT™ Fluorescent Oligo and Stealth™ RNA or standard siRNA oligomers to mammalian cells for RNAi analysis (Gitlin *et al.*, 2002; Yu *et al.*, 2002). Using Lipofectamine™ 2000 to transfect eukaryotic cells offers the following advantages:

- Provides the highest transfection efficiency in a wide variety of mammalian cell types
- Oligomer-Lipofectamine™ 2000 complexes can be added directly to cells in culture medium in the presence of serum
- Removal of complexes or medium change or addition following transfection is not required, although complexes can be removed after 4-6 hours without loss of activity

Lipofectamine™ 2000 is supplied with the BLOCK-iT™ Transfection Kit, but is also available separately from Invitrogen (see page vi for ordering information).

Stealth™ RNA

Stealth™ RNA 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

For more information about Stealth™ RNA, see our Web site (www.invitrogen.com) or contact Technical Service (see page 10). To design and order Stealth™ RNA molecules, see the RNAi Designer on our Web site.

Methods

Handling the BLOCK-iT™ Fluorescent Oligo

Handling the BLOCK-iT™ Fluorescent Oligo

The BLOCK-iT™ Fluorescent Oligo is supplied in this kit as a 20 μM stock solution in an annealing buffer. Follow the guidelines below when handling the BLOCK-iT™ Fluorescent Oligo stock solution.

- The BLOCK-iT™ Fluorescent Oligo 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 on ice or at room temperature. Once thawed, place the tube on ice until use. After use, return the stock solution to -20°C storage.
- The stock solution may be frozen and thawed multiple times without loss of fluorescence signal if handled properly.
- Take precautions to ensure that the stock solution does not become contaminated with RNase.
    a. Use RNase-free sterile pipette tips and supplies for all manipulations.
    b. Wear gloves when handling reagents and solutions.

Guidelines for Transfection

Introduction

General guidelines are provided in this section to use Lipofectamine™ 2000 to transfect the BLOCK-iT™ Fluorescent Oligo into mammalian cells.

General Guidelines for Transfection

Follow these general guidelines when using Lipofectamine™ 2000 to transfect the BLOCK-iT™ Fluorescent Oligo into mammalian cells.

- Determine the appropriate amount of BLOCK-iT™ Fluorescent Oligo to use such that fluorescence signal is readily detectable. For recommended reagent amounts to use, see the next page.

  Note: Once you have determined optimal transfection conditions using the BLOCK-iT™ Fluorescent Oligo, you may use these conditions as a starting point to transfect Stealth™ RNA or siRNA, with optimization as necessary.

- Use low-passage cells, and make sure that cells are healthy and greater than 90% viable before transfection.
- **Transfect cells at 30-50% confluence.** We recommend assessing BLOCK-iT™ Fluorescent Oligo uptake at 6 to 24 hours post-transfection; however, target gene knock-down levels (following Stealth™ RNA or siRNA delivery) are generally assayed at a minimum of 24 to 72 hours following transfection. Transfecting cells at a lower density allows a longer time interval to elapse between transfection and assay time, and minimizes the loss of cell viability due to cell overgrowth. Depending on the nature of the target gene, transfecting cells at higher densities may be suitable with optimization of conditions.
- **Do not add antibiotics to the medium during transfection** as this reduces transfection efficiency and causes cell death.
- For optimal results, use Opti-MEM® I Reduced Serum Medium (Catalog no. 31985-062) to dilute Lipofectamine™ 2000 and dsRNA oligomers prior to complex formation.

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Guidelines for 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 Lipofectamine™ 2000 and vary the final concentration of 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.

If you wish to assess viability of your mammalian cells following transfection of Stealth™ RNA or siRNA, we recommend using the Dead Cell Reagent available from Invitrogen (see page vi for ordering information). Dead Cell Reagent is an ethidium dye that enters cells with damaged membranes and emits a red fluorescence signal upon binding to nucleic acids. Fluorescence signal is readily detected using a fluorescence microscope and filters for propidium iodide or Texas Red®. For more information about Dead Cell Reagent, see our Web site (www.invitrogen.com) or call Technical Service (see page 10).

Transfection Procedure

Introduction

This section provides the following:

- A procedure to transfect the BLOCK-iT™ Fluorescent Oligo into mammalian cells using Lipofectamine™ 2000.
- A table listing recommended BLOCK-iT™ Fluorescent Oligo and Lipofectamine™ 2000 amounts and volumes to use for transfection in different tissue culture formats.

Materials Needed

Have the following reagents on hand before beginning:

- Mammalian cell line of interest cultured in the appropriate growth medium
- BLOCK-iT™ Fluorescent Oligo (supplied with the kit; 20 µM in annealing buffer)
- Lipofectamine™ 2000 Reagent (supplied with the kit; store at +4°C until use)
- Opti-MEM® I Reduced Serum Medium (pre-warm to 37°C before use)
- Appropriate tissue culture plates and supplies

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Transfection Procedure, continued

Transfection Procedure

Use this procedure to transfect the BLOCK-iT™ Fluorescent Oligo into mammalian cells using Lipofectamine™ 2000. Refer to the table in **Suggested Reagent Amounts and Volumes**, next page for the appropriate reagent amounts and volumes to add for different tissue culture formats. Use the recommended amounts as a starting point for your experiments, and optimize conditions for your cell line.

1. One day before transfection, plate cells in the appropriate amount of growth medium **without antibiotics** such that they will be 30-50% confluent at the time of transfection.
2. For each transfection sample, prepare oligomer-Lipofectamine™ 2000 complexes as follows:
   a. Dilute the BLOCK-iT™ Fluorescent Oligo in the appropriate amount of Opti-MEM® I Reduced Serum Medium without serum. Mix gently.
   b. Mix Lipofectamine™ 2000 gently before use, then dilute the appropriate amount in Opti-MEM® I Reduced Serum Medium. Mix gently and incubate for 5 minutes at room temperature.

      Note: Combine the diluted Lipofectamine™ 2000 with the diluted oligomer within 30 minutes. Longer incubation times may decrease activity.

   c. After the 5-minute incubation, combine the diluted oligomer with the diluted Lipofectamine™ 2000. Mix gently and incubate for 20 minutes at room temperature to allow complex formation to occur. The solution may appear cloudy, but this will not inhibit transfection.
3. Add the oligomer-Lipofectamine™ 2000 complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.
4. Incubate the cells at 37°C in a CO_2 incubator until you are ready to assess fluorescent uptake (see **Note** on the next page). Removal of complexes or media change is not required; however, growth medium may be replaced after 4-6 hours without loss of transfection activity.

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Transfection Procedure, continued

Note

We recommend assessing fluorescent uptake at 6 to 24 hours post-transfection. The fluorescence signal may be detected at later time points depending on the transfection efficiency and growth rate of the cells.

Suggested Reagent Amounts and Volumes

The table below lists the recommended reagent amounts and volumes to use to transfect cells in various tissue culture formats. Use the recommended amounts of BLOCK-iT™ Fluorescent Oligo (see column 4) and Lipofectamine™ 2000 (see column 6) as a starting point for your experiments, and optimize conditions for your cell line.

Note: 20 µM BLOCK-iT™ Fluorescent Oligo = 20 pmol/µl.

Culture Vessel	Relative Surface Area (vs. 24-well)	Volume of Plating Medium	Oligo (pmol) and Dilution Volume (µl)	Oligo Amts (pmol) to Optimize	Lipid (µl) and Dilution Volume (µl)	Lipid Amts (µl) to Optimize
48-well	0.4	200 µl	25 pmol in 25 µl	1-50 pmol	0.5 µl in 25 µl	0.3-0.8 µl
24-well	1	500 µl	50 pmol in 50 µl	5-100 pmol	1 µl in 50 µl	0.5-1.5 µl
6-well	5	2 ml	250 pmol in 250 µl	25-500 pmol	5 µl in 250 µl	2.5-6 µl

Detecting Fluorescence Signal

Once you have transfected your mammalian cells with the BLOCK-iT™ Fluorescent Oligo, you may qualitatively assess Oligo uptake using any fluorescence microscope and a standard FITC filter set (λ_{ex} = 494 nm, λ_{em} = 519 green) to detect the fluorescence signal.

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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Technical Service, continued

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Introduction

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Limited Use Label License No. 27: Lipofectamine™ 2000

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Product Qualification

BLOCK-iT™ Fluorescent Oligo

The BLOCK-iT™ Fluorescent Oligo is qualified as follows:

- Concentration of the Oligo is verified by reading the optical density (OD)
- Oligo is functionally qualified by transient transfection into mammalian cells and assessment of fluorescence signal at 24 hours post-transfection

Lipofectamine™ 2000

Lipofectamine™ 2000 is tested for the absence of microbial contamination using blood agar plates, Sabaraud dextrose agar plates, and fluid thioglycolate medium, and functionally by transfection with a luciferase reporter-containing plasmid.

References

Fisher, T. L., Terhorst, T., Cao, X., and Wagner, R. W. (1993). Intracellular Disposition and Metabolism of Fluorescently-Labeled Unmodified and Modified Oligonucleotides Microinjected into Mammalian Cells. Nuc. Acids Res. *21*, 3857-3865.

Gitlin, L., Karelsky, S., and Andino, R. (2002). Short Interfering RNA Confers Intracellular Antiviral Immunity in Human Cells. Nature *418*, 430-434.

Yu, J. Y., DeRuiter, S. L., and Turner, D. L. (2002). RNA Interference by Expression of Short-interfering RNAs and Hairpin RNAs in Mammalian Cells. Proc. Natl. Acad. Sci. USA *99*, 6047-6052.

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