invitrogen™

BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo

Cat. No. 14750-100 **Size: 2 x 125 µl**

Shipping and Storage

The BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo is shipped on dry ice. Upon receipt, store at -20°C, protected from light.

Contents

The BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo is supplied as a 20 µM stock of Alexa Fluor® 555-labeled, double-stranded RNA (dsRNA) oligomer in 100 mM KOAc, 30 mM HEPES-KOH, pH 7.4, and 2 mM MgOAc (annealing buffer).

Description

The BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo is a red-labeled dsRNA oligomer designed for use in RNAi analysis to facilitate assessment and optimization of dsRNA oligonucleotides delivery into mammalian cells using cationic lipids (including Lipofectamine™ RNAiMAX and Lipofectamine™ 2000). Using the BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo in RNAi studies offers the following advantages:

- Gives a good indication of transfection efficiency with Invitrogen's Stealth™ RNAi, standard unmodified siRNA, or purified Dicer-generated siRNA.
- Allows strong, easy fluorescence-based indication of transfection efficiency in every RNAi experiment.

Product Qualification

The BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo is qualified as follows:

- The identity and concentration of each corresponding Alexa labeled single-stranded RNA oligomer is verified by mass spectrometry and optical density reading, respectively.
- After annealing, the BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo is analyzed by PAGE gel electrophoresis to verify its integrity and to confirm the absence of RNA degradation.

For technical support, email tech_support@invitrogen.com.
For country-specific contact information, visit ***www.invitrogen.com***.

Characteristics of BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo

The BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo possesses the following characteristics:

- Is an Alexa Fluor® 555-labeled 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
- The sequence of the BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo is not homologous to any known gene, ensuring against induction of non-specific cellular events caused by introduction of the duplex into cells.

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

Handling the BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo

The BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo is supplied as a 20 µM stock solution in annealing buffer. Follow the guidelines below when handling the BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo stock solution.

1. The BLOCK-iT™ Alexa Fluor® Red 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.
2. 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.
3. The stock solution may be frozen and thawed multiple times without loss of fluorescence signal if handled properly.
4. 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.

Using the BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo

You may use the BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo (20 µM stock) with any cationic lipid-based transfection reagent suitable for delivery of Stealth™ RNAi, siRNA, or Dicer-generated siRNA to mammalian cells. Follow the guidelines below when transfecting the BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo.

Tip: For highly efficient transfection of a wide variety of mammalian cells, we recommend using Lipofectamine™ RNAiMAX (Catalog no. 13778-075) or Lipofectamine™ 2000 Reagent (Catalog no. 11668-027), available from Invitrogen.

- The amount of BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo to use depends on the growth rate and transfection efficiency of the mammalian cells. If you are transfecting your mammalian cell line for the first time, we recommend evaluating several concentrations of lipid and varying the final concentration of the BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo from 10 to 100 nM to determine the optimal amount of BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo to use to obtain a strong fluorescence signal.
- Prepare and seed mammalian cells at a density recommended by the manufacturer of the transfection reagent you are using.
- Prepare BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo transfection complexes as directed by the manufacturer of the transfection reagent you are using. Always dilute the BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo immediately before transfection (i.e. do not store diluted oligo) and into an appropriate medium. We recommend diluting the BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo into Opti-MEM® I Reduced Serum Medium (Catalog no. 31985-062), available from Invitrogen.
- Assess Red Fluorescent uptake at 6 to 24 hours post-transfection. The fluorescence signal may be detected at longer time points depending on the transfection efficiency and growth rate of the cells

Detecting Fluorescence Signal

Once you have transfected your mammalian cells with the BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo, you may qualitatively assess the uptake in live cells using fluorescence microscopy. You may use any type of fluorescence microscope and any standard filter set used for the detection of Texas Red or rhodamime fluorescence. The excitation peak of Alexa Fluor® 555 is 555 nm, and the emission peak is 565 nm. The cell culture medium may be replaced with PBS (Catalog no. 14190-144, or equivalent) for improved fluorescence detection.

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