PRODUCT INSERT

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Dharmacon™ siGLO™ transfection indicators

Please read

siGLO™ transfection indicators (siGLO Green and siGLO Red) are fluorescent oligonucleotides that localize to the nucleus thus permitting unambiguous visual assessment of uptake into mammalian cells. This product is not intended to provide information about siRNA function, localization or duration of silencing. They are ideal[1] for use in experiments to determine optimal siRNA transfection conditions[2] for tracking individually transfected cells and (3) for monitoring relative efficiency of delivery when co-transfected with siRNA.

Product description

- Double-stranded, chemically synthesized RNA
- Chemically labeled with 6-FAM or Cy3 fluorophores (see Table 1)
- Chemically modified to prevent uptake by RISC and assure optimal localization to the nucleus

Table 1. Recommended resuspension buffer volumes and final siRNA concentrations.

siGLO transfection indicator	Fluorophore absorption/emission max	Extinction coefficient	Filter	Cat. #
siGLO Green	6-FAM (494 nM/520 nM)	75,000 M^{-1} cm^{-1}	FITC	D-001630-01-XX
siGLO Red	Cy3 (547 nM/563 nM)	136,000 M^{-1} cm^{-1}	Cy™3, Rhodamine and PE	D-001630-02-XX

XX=05,20, or 50 for 5, 20, and 50 nmol amounts

Shipping and storage

- siGLO transfection indicators are shipped at room temperature (23 °C) as dried pellets in amber tubes. Under these conditions, they are stable for at least four weeks.
- Upon receipt, siGLO transfection indicators should be stored at –20 °C to –80 °C, protected from ambient light. Under these conditions, they are stable for at least nine months.
- siRNA should be resuspended in RNase-free solutions. We recommend 1x siRNA buffer (diluted from 5x siRNA buffer - Cat. #B-002000-UB-100).
- RNase-free water (Dharmacon Cat.#B-003000-WB-100) is also appropriate for resuspension and short-term storage of concentrated stocks (20-100 µM). Alternatively, an RNase-free buffer (pH 7.3–7.6) may be used such as PBS.
- Upon resuspension, aliquot the siRNA into small volumes and store at –20 °C to –80 °C. For best results, limit freeze-thawing of each tube to no more than five events. Under these conditions, the siRNA is stable for at least 6 months

Resuspension

Oligonucleotides are susceptible to enzymatic degradation by nucleases and to chemical degradation by extreme pH and temperature. We recommend wearing gloves and maintaining nuclease-free conditions when handling the oligonucleotides.

1. Briefly centrifuge tubes containing siGLO transfection indicators to ensure that the pellet is collected at the bottom of the tube.
2. Resuspend siGLO transfection indicators to the desired stock concentration in 1x siRNA buffer or another RNase-free, pH 7.4-buffered solution (see Table 2).
3. Incubate at room temperature for 20–30 minutes with gentle mixing to ensure complete resuspension. Centrifuge briefly to collect contents to the bottom of the tube.
4. Upon resuspension, aliquot the siGLO transfection indicators into small volumes and store at –20 °C to –80 °C protected from ambient light.

Table 2. Preparing stock solutions

siGLO transfection indicators (nmol)	Resuspension buffer (mL) for 20 µM	Resuspension buffer (mL) for 100 µM
5	0.25	0.05
20	1.00	0.2
50	2.5*	0.5

*Volume recommended exceeds capacity of tube provided

Guidelines for use

Delivery and detection

siGLO transfection indicators are suitable for delivery into mammalian cells by lipid-mediated transfection, electroporation, or nucleofection. For the best signal, detection should be performed at 24 hours. For microscopy, a 400x magnification with an exposure time of 2.5–5 seconds and a gain of 8 produces good images. Fixing with 4% paraformaldehyde or co-staining with nuclear stains (propidium iodide or Hoechst 33342) does not affect the signal; however, ethanol or methanol-based fixatives may lead to loss of signal.

Due to distinct spectral properties of the two fluorophores, performance may differ based on the delivery and detection methods employed. siGLO Green (FAM) exhibits minimal punctuate fluorescence, with virtually 100% of the nuclear signal localized to the nucleus and represents the ideal choice for microscopy or flow cytometry following lipid-mediated transfection. siGLO Red (Cy3) exhibits a stronger signal at lower concentrations and is best for electroporation or nucleofection.

Recommended concentrations

siGLO transfection indicators are suitable for use alone in delivery optimization experiments or with a functional siRNA for identification of transfected cells for single-cell analysis. When used alone, the recommended concentration range of siGLO Green or siGLO Red is 20 nM-50 nM for lipid-mediated delivery. When co-delivered with a functional siRNA the recommendation is to use a 1:1 ratio not to exceed 100 nM (for example, up to 50 nM each). When performing electroporation or nucleofection, higher concentrations of siGLO transfection indicators will be required. When used alone, the recommended concentration range of siGLO Green or siGLO Red is 500 nM-2 µM.

The siGLO transfection indicators should not be used at concentrations less than 20 nM as it will be difficult to visualize.

Note that optimal siRNA concentrations should be determined based on function (for example, titrate the siRNA to the lowest concentration that delivers the best knockdown).

Related products

DharmaFECT™ Transfection Reagents are available in four formulations that are optimized for transfecting siRNA into a wide variety of cell lines. For more information, click here.

Publication reference guide

When referencing the use of the siGLO transfection indicators described in this document, please include the following information: siGLO Transfection Indicator (Red or Green), Cat #, Horizon Discovery (Dharmacon), Lafayette, CO.

For more information

To find the contact information in your country for your technology of interest, please visit us at **horizondiscovery.com/contact-us**

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