Stealth™ RNAi Reporter Control Duplexes

Cat. No. 12935-145	**GFP Reporter Control**
Cat. No. 12935-146	**Luciferase Reporter Control**
Cat. No. 12935-147	**LacZ Reporter Control**
Cat. No. 12935-148	**β-lactamase Reporter Control**

Description

Stealth™ RNAi Reporter Control Duplexes are ideal for use in RNA interference (RNAi) experiments to help you optimize your transfection conditions in any vertebrate cell line. Each Stealth™ RNAi Reporter Control Duplex is designed to efficiently knock-down its intended target and minimize sequence homology to any other known vertebrate transcript. The Stealth™ RNAi Reporter Control Duplexes are supplied in a ready-to-use format and 1X RNA Annealing/Dilution Buffer is included for dilution of the Stealth™ RNAi stock solution, if desired.

Components

Each Stealth™ RNAi Negative Control Duplex includes the appropriate Stealth™ RNAi Reporter Control Duplex and 1 ml of 1X RNA Annealing/Dilution Buffer, with the following compositions:

Item	Composition	Amount
Stealth™ RNAi Reporter Control Duplex	20 µM in 1X RNA Annealing/Dilution Buffer	250 µl
1X RNA Annealing/Dilution Buffer	10 mM Tris-HCl, pH 8.0 20 mM NaCl 1 mM EDTA, pH 8.0	1 ml

Storage

Store the Stealth™ RNAi Reporter Control Duplexes and the 1X RNA Annealing/Dilution Buffer at -20°C.

Part No.: 12935.rep.pps Rev. Date: 19 April 2007

Stealth™ RNAi

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

- Obtain effective target gene knockdown
- Eliminates sense strand off-target effects for higher specificity
- Exhibits enhanced stability for greater flexibility in RNAi analysis
- Avoids induction of cellular stress response pathways

For more information about Stealth™ RNAi, see www.invitrogen.com/rnai.

Stealth™ RNAi Reporter Control Duplex Target Genes

The Stealth™ RNAi Reporter Control Duplex target the following genes:

- **Stealth™ RNAi GFP Reporter Control** targets the following fluorescent proteins: Emerald Green Fluorescent Protein (EmGFP), Enhanced GFP, Enhanced Cyan Fluorescent Protein (ECFP),Yellow Fluorescent Protein (YFP), and Topaz fluorescent protein (Tpz).
- **Stealth™ RNAi Luciferase Reporter Control** targets the firefly (*Photinus pyralis*) luciferase gene, commonly used in reporter vectors such as pGL2 and pGL3. The Stealth™ RNAi Luciferase Reporter Control does not knock-down luciferase genes from other species, such as *Renilla* luciferase.
- **Stealth™ RNAi LacZ Reporter Control** targets the *E. coli LacZ* gene, encoding β-galactosidase, which is a frequently used reporter gene and fusion protein.
- **Stealth™ RNAi β-lactamase Reporter Control** targets the *bla* gene encoding the β-lactamase enzyme, a regularly used reporter gene, for example in the GeneBLAzer® Technology from Invitrogen.

Handling the Stealth™ RNAi Reporter Control Duplexes

- Thaw Stealth™ RNAi Reporter Control Duplex stock solutions on ice or at room temperature. After use, return to -20°C storage.
- Multiple freeze/thaw cycles are permitted without loss of activity if stock solutions are handled properly.
- Ensure that the stock solutions do not become contaminated with RNase.

Amount of Stealth™ RNAi Reporter Control Duplex to Transfect

The amount of Stealth™ RNAi duplex or the Stealth™ RNAi Reporter Control Duplex required to achieve optimal target gene knockdown or minimal knockdown, as appropriate, should be determined experimentally for each human cell line. **As a starting point, we recommend using 10 nM Stealth™ RNAi duplex or Stealth™ RNAi Reporter Control Duplex for transfection.**

To optimize transfection conditions, vary transfection reagent concentrations and the final concentrations of Stealth™ RNAi from 1 to 200 nM, as necessary for your cell line. Use the 1X RNA Annealing/Dilution Buffer supplied with the kit to dilute the Stealth™ RNAi Reporter Control Duplex stock solution, if necessary.

General Guidelines for Transfection

- Use a transfection reagent suitable for delivery of Stealth™ RNAi to mammalian cells. **For optimal results, we recommend using Lipofectamine™ RNAiMAX (Cat. No. 13778-075) available from Invitrogen.** For a protocol to transfect Stealth™ RNAi into cells using Lipofectamine™ RNAiMAX, see the RNAi resource page at www.invitrogen.com/rnai; click on Protocols.
- Use cells that express the appropriate target gene for the Stealth™ RNAi Reporter Control Duplex you are using (see **Stealth™ RNAi Reporter Control Duplex Target Genes**), for example through stable integration or viral transduction of the reporter construct. Alternatively, co-transfect the Stealth™ RNAi duplex with the appropriate reporter expression construct.

  Note: For co-transfection experiments with a plasmid, we recommend using Lipofectamine™ 2000, since Lipofectamine™ RNAiMAX was specifically designed for use with Stealth™ RNAi or siRNA duplexes
- Use low-passage cells, and make sure that cells are healthy and greater than 90% viable before transfection.
- Transfect cells at the density recommended by the manufacturer of your transfection reagent (*e.g.* 30-50% confluence at time of transfection if using Lipofectamine™ RNAiMAX).
- Assay for target gene knockdown at a suitable time period (typically 24 to 72 hours) after transfection.
- For a convenient tool to assess transfection efficiency and Stealth™ RNAi uptake, we recommend using the BLOCK-iT™ Alexa Fluor® Red Fluorescent Control (Cat. No. 14750-100) available from Invitrogen.

Quality Control

- The identity and concentration of each corresponding single-stranded RNA oligo is verified by mass spectrometry and optical density reading, respectively.
- After annealing, the Stealth™ RNAi Negative Control Duplex is analyzed by polyacrylamide gel electrophoresis to verify its integrity and to confirm the absence of RNA degradation.

Limited Use Label License No. 196: Stealth™ RNAi

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