# Fluorescein-siRNA Transfection Control

1-800-632-7799
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www.neb.com

N2100S 008120914091

# N2100S

**100 transfections (12-well format)** **Lot: 0081209**

**1 nmol** **Store at –20°C** **Exp: 9/14**

**Description:** The fluorescein labeled siRNA is used as a control for RNA transfections. The chemically synthesized 21 base RNA has two complementary strands each with a fluorescein linked to the 5´ end base. It has a 19 bp dsRNA region with 2 base 3´ extensions.The sequence of Fluorescein-siRNA Transfection control has no sequence identity to any mammalian sequences in the database.

**Protect from light**

Visible Fluorescein

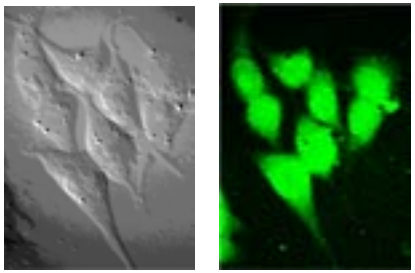

***Figure 1:*** *HCT116 cells transfected with Fluorescein-siRNA Transfection Control using TransPass™ R1 Transfection Reagent (NEB #M2551S).*

The sequences of the two complementary strands are given below:

Fluorescein-5´AGGUCGAACUACGGGUCAAUC3´
MW: 7272.6 g/ml

Fluorescein-5´UUGACCCGUAGUUCGACCUAG3´
MW: 7186.5 g/ml

The Fluorescein-siRNA can be used to estimate the transfection efficiency of siRNA with a particular cell line. It facilitates parameter optimization such as cell density, amount of transfection reagent, etc.

**Recommended Usage Concentration:** 15 nM

Supplied at a concentration of 10 µM in: 20 mM KCl, 10 mM Na-HEPES (pH 7.0) and 0.5 mM EDTA (ready for transfection).

**Tested in the following cell lines:** HeLa, COS-7, NIH3T3, HCT116.

**Quality Assurance:** Purified by gel electrophoresis.

**Transfection of siRNA Mixtures:**
The transfection of siRNA must be optimized in order to obtain maximum silencing of a target gene. Optimizing the following parameters may be necessary in order to maximize the transfection efficiency for a particular cell line: the cell density at the time of transfection, the amount of transfection reagent, the amount of siRNA and the culture incubation time before analysis.

**Suggested Protocol:**
Values given are for a 12-well plate. Values for different sized plates are listed in **Table I**.

1. Plate cells on a 12-well plate at an appropriate density so that they will reach 40–50% confluence at the time of transfection.
2. Mix 4 µl of TransPass™ R1 Transfection Reagent (NEB #M2551) in 100 µl of serum-free medium (e.g., High Glucose DMEM) in a sterile tube and mix by vortexing. Incubate at room temperature for 10–20 minutes.
3. Add 1–2 µl of Fluorescein siRNA control[1] to the diluted transfection reagent, mix gently by pipetting and incubate 10–20 minutes at room temperature to form the transfection complexes.
   [1] The concentration of siRNA Mix is 10 µM = 144.6 ng /µl.
4. Dilute the complex with 500 µl of complete culture medium, (e.g., 10% FBS-containing DMEM). (The final concentration of siRNA will be 15 nM).
5. Aspirate the culture medium from the cells and immediately replace with the diluted transfection complex misture. Evenly disperse the siRNA complexes by gently rocking the plate.

*(See other side)*

CERTIFICATE OF ANALYSIS

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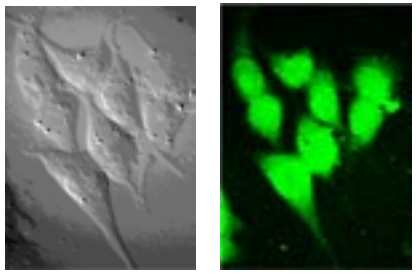

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(See other side)

CERTIFICATE OF ANALYSIS

Typical cell incubation time points for detecting Fluorescein siRNA uptake is 4–8 hours after transfection.

**Table I. siRNA Transfection**

Volumes are shown for one transfection per well for the indicated plate size. For the listed volumes shown, the range of siRNA concentration will be 2.5–25 nM.

| Plate Size | 6 | 12 | 24 | 96 |
|---|---|---|---|---|
| TransPass R1 siRNA Transfection Reagent | 4–8 µl | 2–6 µl | 1–4 µl | 0.5–2 µl |
| Serum Free Medium | 200 µl | 100 µl | 50 µl | 25 µl |
| siRNA Mix (10 µM) | 0.3–3 µl | 0.15–1.5 µl | 0.1–1 µl | 0.03–0.3 µl |
| Complete Medium | 1000 µl | 500 µl | 350 µl | 100 µl |
| Final Volume | 1.2 ml | 0.6 ml | 0.4 ml | 0.125 ml |



**Notes:**

1. It is important to maintain healthy cells. Some cell lines become more sensitive to transfection agents after a large number of passages. It is advisable to use cells subjected to a similar number of passages to ensure reproducible transfection results in different experiments.
2. It is recommended that control transfections be performed by varying cell confluence and using different amounts of TransPass Transfection Reagents. In general, low cell density or too much transfection reagent increase the risk of cell toxicity. The medium may be replaced 24 hours after transfection with fresh complete medium to increase cell viability.

   In order to easily estimate the efficiency of transfection of particular cell lines use the Fluorescein-siRNA Transfection Control (NEB #N2100).
3. TransPass™ R1 Transfection Reagent has been used to successfully transfect siRNAs in many cell lines including: A549, C6, CHO, COS-7, HEK293, NIH3T3, HepG2, HCT116, HeLa, Jurkat, MCF-7, U2OS and 3T3-L1 preadipocytes.

**Companion Products:**

TransPass™ R1 Transfection Reagent
#M2551S 0.4 ml

TransPass™ R2 Transfection Reagent
#M2552S 1.0 ml

Lit28i Polylinker ShortCut siRNA Mix
#N2014S 1 nmol

MBP Control ShortCut siRNA Mix
#N2017S 1 nmol

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