Users Guide: FuGENE® SI Transfection Reagent

For the transient transfection of RNAi molecules (siRNA, miRNA & similar) into eukaryotic and insect cells

For life science research only

Cat. No SI-1000 1ml

1. What this Product Does

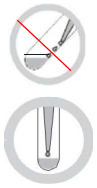
Formulation

FuGENE® SI Transfection Reagent is a 100% synthetic, proprietary blend of lipids and other components engineered for high efficiency and low toxicity transfection of RNA molecules into eukaryotic cells. It is supplied in 60-70% ethanol, 0.1um sterile-filtered, and packaged in glass-vials. It does not contain any ingredients of human or animal origin.

Storage and stability

FuGENE® SI Reagent is shipped at room temperature. FuGENE® SI Transfection Reagent is stabilized for extended storage at +2 to +8°C through the expiration date printed on the label when very tightly closed. Always bring to room temperature and mix FuGENE® SI Transfection Reagent prior to use.

Special Handling



Do not aliquot FuGENE® SI Reagent from the original glass vials. Chemical residues in plastic vials can significantly decrease the biological activity of the reagent. Minimize the contact of undiluted FuGENE® SI Reagent with plastic surfaces. Always dilute the reagent by pipetting directly into serum- free medium. Do not allow the FuGENE® SI Reagent to contact the plastic walls of the tube containing the serum-free medium during the dilution step.

Note: FuGENE® SI Transfection Reagent remains fully functional even after repeated vial openings as long as the vials are tightly recapped and stored at +2 to +8°C between uses.

1.1 Product overview

Number of transfection experiments

In a typical experiment 1 mL of FuGENE® SI Transfection reagent can be used to perform up to 150 transfections in 35-mm dish utilizing 7.5ul of reagent combined with 25 pmol of siRNA per well. This is equivalent to over 3,333 wells in 96-well plate or 666 wells in 24-well plate.

- Note: Optimal transfection and knockdown depends upon experimental conditions including cell type, passage history, confluence, seeding protocol, complex incubation time, serum batch, etc. The above amounts of reagents work well with HEK293 cell lines. In other systems, increased amounts of siRNA and FuGENE® SI may yield optimal levels of knockdown.

Quality control

Functional analysis

0.3ul of FuGENE® SI Transfection Reagent is combined with 1 pmol of GFP targeting siRNA or 1pmol of scrambled negative control, and used to transfect GFP stable expressing HEK293, CHOK1, and HeLa labeled cells in the presence of 10% fetal bovine serum (FBS). Following transfection, gene knockdown is assessed after 48 hours via flow cytometry.

Cytotoxicity analysis

HEK-293/GFP cells that are continuously exposed to FuGENE® SI Reagent for 48 hours, with or without RNA, in the presence of serum, and without a change of medium, are >90% viable by flow-cytometric analysis based on propidium-iodide staining.

1.2 Background Information

Application:

FuGENE® SI Transfection Reagent is a multi-component reagent that forms a complex with siRNA, miRNA, and other small RNAs, and then safely and efficiently transports it into eukaryotic cells. Benefits of FuGENE® SI Reagent include:

- Superior knockdown efficiency while being gentle on cells.
- High transfection efficiency in many common cell types and difficult-to-transfect cell lines.
- Minimal-to-no cytotoxicity, allowing you to work with fewer cells, and eliminates the requirement to change media.
- Requires minimal optimization.
- Flexible and quick protocols

RNA Interference:

FuGENE® SI was designed for the delivery of siRNA, miRNA, and other small RNA's into routine and difficult-to-transfect cell lines. Use a 10nM siRNA as a starting point.

Cell-culture conditions

Minimize variance in transfection efficiency by using cells that are regularly passaged, proliferating well (best when in a log-growth phase), and plated at a consistent density.

Other media additives

In some cell types, antimicrobial agents (*e.g.*, antibiotics and fungicides) that are commonly included in cell-culture media may adversely affect the transfection efficiency of FuGENE® SI Transfection Reagent. If possible, exclude additives for initial experiments. Once high-efficiency conditions have been established, these components can be added back while monitoring your transfection results.

Incubation Time

Incubate the cells for 24–72 hours. The length of incubation depends upon the siRNA gene target, the cell type being transfected, and the downstream assay being utilized. After this incubation period, measure gene or protein expression using an assay that is appropriate for your system.

1.3 Procedures and Required Materials

Before you begin

Additional required reagents and supplies

1.) Sterile, serum-free DMEM culture medium without additives or supplements.
2.) siRNA, miRNA, or related small RNAs.

Preparation of cells for transfection: Choose an option below

Option 1: Rapid Forward Protocol: The day of transfection, plate cells (typically 2x the amount cells that would be utilized in a traditional protocol where cells are plated the day before transfection.) Proceed directly to transfection

Option 2:Traditional Forward Protocol: One day before the transfection experiment, trypsinize cells, adjust the cell concentration, and plate the cells. Place cells in incubator overnight.

Option 3: Reverse (High-through put screening) Protocol: Prepare FuGENE SI and siRNA complex in assay plate as directed, add cells directly to assay plate containing the complex (typically 2x the amount of cells that would be utilized in a traditional forward protocol).

Please reference Table 1 for recommended starting cell seeding densities.

2.) How to use this product

The outlined protocol will generate a master-mix enough to transfect a single 35-mm culture dish, one well of 6-well plate, five wells of a 24-well plate, or twenty-five wells of a 96-well plate at the recommended starting ratio (0.3ul FuGENE SI + 1pmol siRNA per well of 96-well plate). For further scaling and other vessel sizes please see Table 1.

1.) Allow FuGENE® SI Transfection Reagent, RNA, and media to adjust to room temperature prior to usage. Vortex for one second, or invert FuGENE® SI Transfection Reagent vial to mix.

2.) Dilute FuGENE® SI Reagent and siRNA in two separate tubes with serum-free media (without antibiotics or fungicides):

Label two tubes: 1.) "FuGENE® SI" 2.) "siRNA".

Tube 1: Prepare 125ul of diluted FuGENE® SI by pipetting 7.5ul of FuGENE® SI into 117.5ul of media. Immediately tap the tube or vortex for 1 second to mix.

Tube 2: Prepare 125ul of 200nM siRNA by pipetting 2.5ul of 10uM siRNA into 122.5 ul of media. Tap tube or vortex to mix.

3.) Form the FuGENE® SI and siRNA complex:

Add 125ul of diluted siRNA (Tube 2 "siRNA") to 125ul of diluted FuGENE® SI (Tube 1 "FuGENE SI") to make 250ul total volume of complex solution. Tap tube or vortex for 1 second to mix.

4.) Incubate the siRNA-FuGENE® SI complex:

Incubate the siRNA-FuGENE® SI complex for 5 minutes at room temperature. (up to 15 minutes)

5.) Add siRNA-FuGENE® SI complex to the cells:

Remove culture vessel from the incubator. Removal of growth medium is not necessary. Add the siRNA-FuGENE® SI complex to the cells in a drop-wise manner. Swirl the wells or flasks to ensure distribution over the entire plate surface.

35-mm vessel: 250ul added to well (Final amount used per well: 25 pmol siRNA + 7.5 ul FuGENE® SI)
24-well plate: 50ul added per well (Final amount used per well: 5 pmol siRNA + 1.5ul FuGENE® SI)
96-well plate: 10ul added per well (Final amount used per well: 1 pmol siRNA + 0.3ul FuGENE® SI)

See Table 1 for details on the amount of complex to add to other vessel size.

6.) Incubate cells for 24-72 hours. Then, analyze transfected cells via chosen method

Notes:

- As with any experiment, include appropriate controls. Prepare wells with cells that remain non-transfected, cells with transfection reagent alone, and wells with the appropriate positive and negative assay controls.
- The optimal ratio of siRNA to FuGENE® SI, as well as the optimal total amount of complex to add may vary with cell line, cell density, day of assay, and gene target. We recommend testing between 0.1-10.0 pmol siRNA and 0.15-0.6 ul FuGENE® SI per well of a 96-well plate. See optimization guide located in Documents & Manuals section of www.fugene.com

Table 1: Guidelines for Preparing FuGENE® SI + siRNA Complex for Various Culture Vessel Sizes

Table 1 highlights starting amounts for various vessel sizes utilizing the 1pmol siRNA + 0.3ul FuGENE® SI recommended starting ratio for a single well of a 96-well culture vessel. Please note that this is only a starting point, and optimization of siRNA + FuGENE® SI ratio, and total amount of complex to add per well, may be required for specific cell lines and applications.

Culture Vessel	Total Volume Growth Media	Suggested Seeding Density (Adherent Cells)		Volume of FuGENE SI & siRNA complex to add per well	siRNA final amount used (pmol)	FuGENE SI final amount used (ul)
		low	high			
96-well plate (1 well)	100ul	5,000	30,000	10ul	1	0.3
24-well plate (1 well)	500ul	25,000	150,000	50ul	5	1.5
12-well plate (1 well)	1mL	50,000	300,000	100ul	10	3
35-mm dish, or 6-well plate (1 well)	2.5mL	125,000	750,000	250ul	25	7.5
60-mm dish	5mL	250,000	1,500,000	500ul	50	15
10-cm dish	10mL	750,000	4,500,000	1mL	150	45

These are suggested seeding densities and are media, passage level, laboratory, and cell-line dependent. It is critical that log phase cultures are selected for subculture for the transfection experiments, and that cultures are seeded at the proper density for the transfection experiment. Observe cultures and plate them so that the monolayer is 25-50% confluent at the time of transfection.

Troubleshooting/Support:

For information on specific cell line protocols, troubleshooting, or technical support please contact our technical team at:

contact@fugene.com

Notice to Purchaser:

Purchaser represents and warrants that it will use FuGENE® SI Transfection Reagent purely for research purposes and will not attempt to reverse engineer the product. Transfected cells, materials produced, and any data derived from the use of FuGENE® SI Transfection Reagent, may be used only for the internal research of Purchaser whether Purchaser is a "for-profit" or a "not-for-profit" organization. Under no circumstances may FuGENE® SI Transfection Reagent be used by Purchaser or any third party for a commercial purpose unless purchaser has negotiated a license for commercial use with Fugent, LLC (contact information: contact@fugene.com). For purposes of the foregoing sentence, "commercial purpose" shall mean use of FuGENE® SI Transfection Reagent for profit or commercial gain. By using FuGENE® SI Transfection Reagent, Purchaser agrees to be bound by the above terms. If Purchaser wishes not to be bound by these terms, Purchaser agrees to return the FuGENE®SI Transfection Reagent to Fugent LLC. for a full refund.

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